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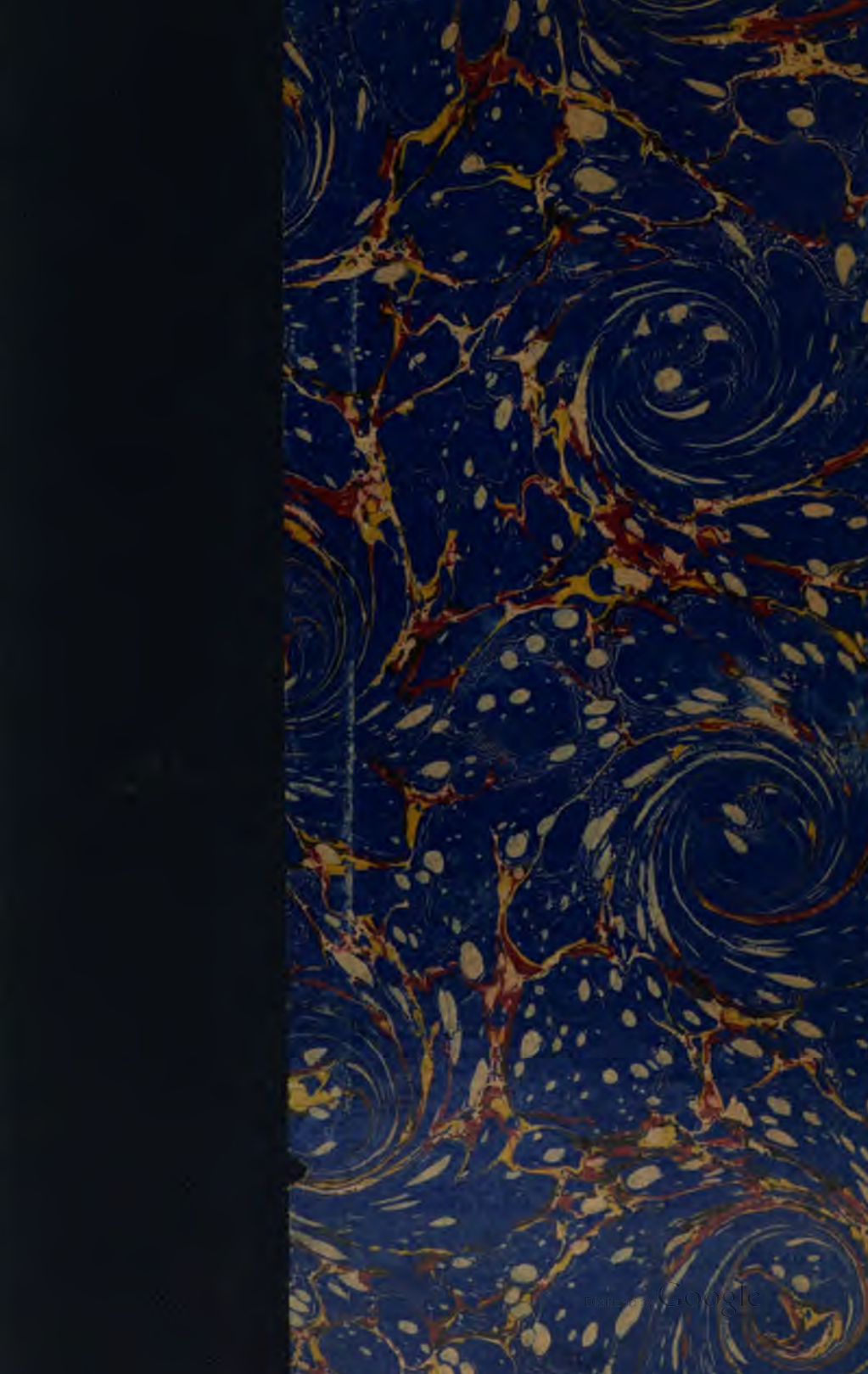
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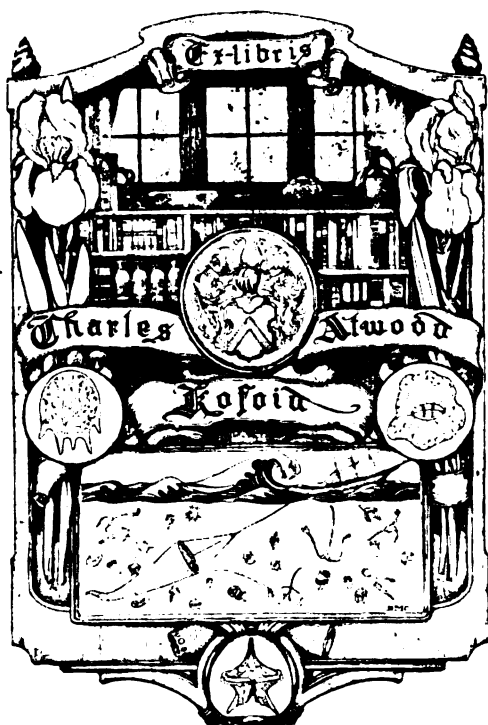
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PRESENTED BY
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REPORTS
OF THE
COMMISSION
APPOINTED BY
THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,
FOR THE INVESTIGATION OF
MEDITERRANEAN FEVER,
UNDER THE SUPERVISION OF AN
ADVISORY COMMITTEE
OF
THE ROYAL SOCIETY.

PART I.

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INTRODUCTION.

The Mediterranean Fever Commission had its origin in a letter from Mr. Secretary Lyttelton, dated January 25, 1904, addressed to the Royal Society, in which he states that his attention has recently been called to the prevalence of Mediterranean fever in Malta among the Naval and Military forces, as well as the civil population.

It accordingly appeared to him to be desirable that the investigation of this fever should be taken in hand, and he addressed a despatch to the Governor of Malta proposing the appointment of a joint Commission representing the Army, the Navy, and the Civil Government.

He enclosed a copy of a despatch from the Governor in reply, entirely concurring in the proposed appointment of a joint Commission. The War Office and Admiralty also expressed their concurrence in the proposal.

Mr. Secretary Lyttelton then went on to say that the War Office, the Admiralty, and the Civil Government desired to secure for this Commission the assistance of the Royal Society, and asked whether the Society would be willing to appoint an Advisory Board of experts in this country for the purpose of supervising the investigations.

In reply to this letter the Royal Society wrote, in February, 1904, consenting to nominate a Committee to direct the investigations, on the understanding that the selection of the investigators should be placed in the hands of the Royal Society.

A Sub-Committee of the Tropical Diseases Committee was accordingly formed, consisting of Colonel Bruce, R.A.M.C., Chairman, Fleet Surgeon Bassett-Smith, R.N., Dr. Klein, Dr. C. J. Martin, and Dr. Sidney Martin.

As it was desirable to begin the investigations with as little delay as possible, the Sub-Committee at once appointed Major Horrocks, R.A.M.C., Staff-Surgeon Shaw, R.N., and Dr. Zammit, Board of Health, Malta, as members of the Commission, and Colonel Bruce was requested to proceed to Malta to assist them in commencing the work. Colonel Bruce arrived in Malta on June 13, where he met the Commission, and work was at once begun. He remained in Malta until July 14, when he left for England. Dr. Johnstone, whose services were lent by the Local Government Board, on the application of the Royal Society, joined the Commission on June 30.

The best thanks of the Commission are due to the Governor, General Sir C. M. Clarke, G.C.B., and to the Lieutenant-Governor, the Hon. E. M. Merewether, C.M.G., for their courtesy and invaluable aid.

The following reports have been received, up to the present date, from the members of the Commission, and also one from Staff-Surgeon Gilmour, R.N., Bigli Hospital, Malta, who kindly placed his spare time at the service of the Commission.

1.

ON THE DURATION OF LIFE OF THE *MICROCOCCUS MELITENSIS* OUTSIDE THE HUMAN BODY.

(Experiments made at Gibraltar.)

By Major W. H. HORROCKS, R.A.M.C., Member of Mediterranean Fever Commission.

(Received July 14, 1904.)

The small size and slow growth of the *Micrococcus melitensis* render the study of its saprophytic existence by no means an easy matter. In the hope of devising a medium which would simplify the isolation of the *Micrococcus* from a mixture of microbes, a careful study of its cultural characteristics on all modern media of an exact reaction was first made. It was thought that the degree of fermentation or non-fermentation of the various sugars might assist in attaining the desired differentiation. The results of the tests are shown in the following table:—

Cultural Characteristics.

<i>Glucose peptone</i> , 1 per cent. . . .	Growth. Neither acid nor gas produced.
<i>Lactose peptone</i> , " . . .	" " "
<i>Saccharose peptone</i> , 1 per cent. . . .	" " "
<i>Starch peptone</i> , 1 per cent. . . .	" " "
<i>Litmus milk</i>	No clotting observed; at the end of 3 weeks the medium was found to have a distinctly alkaline reaction.
<i>Peptone and salt solution</i>	On the addition of a nitrite and pure sulphuric acid, the nitroso-indol reaction was never obtained.
<i>Broth</i>	Diffuse growth without any surface pellicle. After some days the broth cleared somewhat, and a deposit formed on the sides and at the bottom of the tube.
<i>Agar slope</i>	Greyish-white moist growth; discrete colonies, circular and transparent, resembling those of the Gram-staining streptococci found in feces and urine. When the cultures are old, the growth often acquires a yellowish-brown colour.

<i>Proskauer and Capaldi's media</i>	No. I. No growth. No. II. Growth, but no change appeared in the reaction of the medium.
<i>Neutral red</i>	Unchanged after 48 hours at 37° C.; after 5 days' incubation a yellow colour appeared at the surface.
<i>Potato</i>	Moist transparent film appeared, and on scraping the surface a copious growth was obtained, the formation of chains being very marked. The reaction of the potato was made faintly alkaline by the addition of sodium carbonate, and on planting out on the surface a distinct yellowish coloured growth was obtained.
<i>MacConkey's bile salt broth</i> ...	Growth; reaction unchanged.
<i>Nitrate broth</i>	Growth, but no reduction of the nitrates occurred.
<i>Gelatine stab and slope</i> (22° C.)	Growth extremely slow; no liquefaction of the medium.
<i>Agar stab</i> (37° C)	Diffuse growth.
<i>Anaërobiosis</i>	Growth, but more feeble than under aerobic conditions.
<i>Morphology</i>	Very small cocci, appearing as diplococci and short chains; occasionally chains of twelve to fourteen cocci were observed.

The failure of the *M. melitensis* to ferment glucose, and its power of rendering milk alkaline are very important cultural reactions. The Gram-staining streptococci, isolated from sewage, urine, faeces, cases of erysipelas, and from septic throats, all ferment glucose; the amount of acid produced, however, is a variable quantity. In glucose agar media, tinted with litmus, the Gram-staining streptococci produce colonies varying in tint from a rose red to a bright red, but the colonies of the *M. melitensis* are always blue, and after a few days' incubation the colour deepens in tint.

The gelatine, broth, agar, and peptone media, were made with a reaction of + 10 (Eyre's scale), and as a rule distinct growth was not observed until the 2nd or 3rd day after planting out, incubation being at 37° C.

Several observers having stated that the *M. melitensis* grew best on media having an alkaline reaction; batches were prepared having reactions: - 15, - 10, neutral, + 10, + 15 (Eyre's scale). Approximately, the same amount of culture was planted out, and it was found that the quickest and most copious growth was obtained on the + 10 medium; on the - 10 and - 15 there was practically no growth.

Having determined the most favourable reaction, trials were made to see if a medium could not be obtained on which the *M. melitensis* would grow in 24 hours. Bearing in mind the favourable effect of nutrose on the growth of *B. typhosus*, a 1-per-cent. nutrose agar, + 10,

was prepared, and on this a marked growth of *M. melitensis* occurred in 16 hours. A similar vigorous growth was obtained in nutrose broth.

The study of the cultural reactions having shown that the *M. melitensis* did not ferment glucose, it appeared that the addition of this sugar to the nutrose medium, tinted with litmus, would be of great service when isolating the organism from a mixed culture. As previously stated, the Gram-staining streptococci, which occur in urine and faeces, ferment glucose, forming enough acid to change the blue medium to a rose tint, and as the colonies of these organisms have much the same transparent appearance as those of *M. melitensis* on nutrose agar, the use of the glucose litmus medium enables a separation to be readily made, and saves much time when investigating plate cultures.

Trials were then made with the *M. melitensis* added to non-sterile water and soil, and it was found that the organism could be readily isolated when it was present in considerable quantity; when, however, only a few cocci were present, there was a marked tendency for the water and soil organisms to grow over the plate, the nutrose evidently accelerating the growth of these organisms. Accordingly, attempts were made to restrain the growth of these organisms by the addition of sodium taurocholate, carbolic acid, malachite green, etc.

A medium containing 0.5 per cent. sodium taurocholate, 1 per cent. peptone and 0.5 per cent. salt was prepared, and the tubes inoculated with *M. melitensis*, urine, soil, and water respectively. The results are shown in the following table:—

	24 hours.	48 hours.	72 hours.	96 hours.
Tube 1. <i>M. melitensis</i>	±	±	+	+
Tube 2. One loop urine	±	±	+	+
Tube 3. One loop of soil	±	±	+	+

Note.—±, feeble growth; +, good growth; —, no growth.

The growth which appeared in Tube 1, after 48 hours' incubation, was planted out on nutrose agar, and the *M. melitensis* recovered after 3 days' incubation at 37° C.

This experiment showed that, while the sodium taurocholate restrained the growth of the microbes in soil and urine, it had also a marked inhibiting effect on the growth of the *M. melitensis*.

The addition of nutrose to the taurocholate medium was then tried, with the following result:—

	24 hours.	48 hours.	72 hours.
Tube 1. <i>M. melitensis</i>	±	+	+
Tube 2. One loop of urine ...	+	+	++
Tube 3. One loop of soil	±	+	+

The growth in Tube 1, which appeared in 48 hours, was planted out on nutrose agar, and the *M. melitensis* recovered after 48 hours' incubation at 37° C.

The addition of the nutrose caused a more vigorous growth of the *M. melitensis*, but unfortunately the growth of the bacteria in urine was enhanced more than that of the *M. melitensis*. The results with these media when grown at 37° C. being unsatisfactory, the temperature of incubation was raised to 42° C. in the hope that it might cause a more satisfactory separation. Hughes, in his book on Mediterranean fever, stated that the *M. melitensis* would not grow at 42° C., so a preliminary planting out on ordinary agar and nutrose agar was tried. The results were as follows:—

	24 hours.	48 hours.	72 hours.	96 hours.	5 days.
Ordinary agar (+10)	—	—	—	±	±
Nutrose agar (+10)	±	±	±	+	+

Temperature of incubation, 42° C.

On ordinary agar the growth was much delayed and feeble at the end of 5 days, but on nutrose agar a good growth was obtained after 72 hours.

Nutrose, sodium taurocholate peptone tubes were now inoculated with soil, urine, tap-water and *M. melitensis*. Incubation 42° C.

	24 hours.	48 hours.	72 hours.	96 hours.
Tube 1. One c.c. tap-water ..	—	±	±	+
Tube 2. One loop soil	—	±	+	++
Tube 3. One of urine	±	+	+	++
Tube 4. One of <i>M. melitensis</i> .	±	±	±	+

The results were again disappointing; the method would be of very little use in regard to urine investigation, but might render some assistance when working with inoculated water supplies.

Malachite green, krystal violet, etc., being credited with the power of restraining the growth of saprophytes, the former salt was selected for experiment.

The powder was dissolved in distilled water and the solution added to + 10 broth, so as to make dilutions of 0·01 per 1,000, 0·02 per 1,000, 0·05 per 1,000, 0·1 per 1,000, and 0·2 per 1,000. The tubes were incubated at 37° C. for 24 hours, and remaining quite sterile were each inoculated with one loopful of an emulsion of *M. melitensis*. After 24 hours' incubation at 37° C., it was found that there was a good growth of *M. melitensis* in all the tubes except the 0·2 per 1,000. Similar dilutions were then inoculated with urine and soil—the tube containing 0·1 per 1,000 was found to have a marked restraining influence on the growth of the bacteria for a period of 24 hours; but after 48 hours' incubation there was a rapid growth of the bacteria in urine.

Nutrose was then added to the malachite green solution, so that the medium now contained 1 per cent. of nutrose and 0·1 per 1,000 of malachite green.

The tubes were inoculated with an emulsion and incubated at 37° C. After 24 hours it was found that there was a vigorous growth of the *M. melitensis*, but unfortunately, as in the case of the sodium taurocholate, the bacteria in the urine and soil also showed a marked growth. Consequently, it was decided to omit the nutrose from the malachite green broth during the preliminary investigations. A non-sterilised garden soil was inoculated with *M. melitensis* and then planted out in malachite green broth; after 24 hours' incubation at 37° C. a feeble growth occurred, which was stroked over the surface of a series of Petri dishes containing nutrose agar. The plates were incubated at 37° C.; after 24 hours there was practically no growth, but after 48 hours there was a marked growth, and the transparent colonies of the *M. melitensis* were easily detected scattered amongst the larger and opaque colonies produced by the soil organisms. This result was satisfactory, and the procedure appears likely to give useful results.

Carbolic acid was next tried; it was found that the *M. melitensis* grew well in 24 hours in 0·05 per cent. carbolic broth, but this small amount of acid has a very slight restraining influence on the growth of the bacteria in urine and soil, and consequently the *M. melitensis* was always crowded out by the saprophytic bacteria. The amount of carbolic acid was increased to 0·1 per cent., but in this the *M. melitensis* did not appear for 4 days, whereas the saprophytic organism grew vigorously in 48 hours. Accordingly, carbolised media were abandoned during the research.

Exposure to a temperature of 42° C., and the presence of malachite green, carbolic acid and sodium taurocholate, having failed to restrain the growth of bacteria present in urine obtained from Malta fever

patients after careful sterilisation of the external parts, growth under anaërobic conditions was tried but with equally unsatisfactory results. It now appeared evident that in the study of urine all restraining influences must be abandoned and efforts made to obtain as free a growth of the microbes as possible, trusting to subsequent dilution to obtain isolated colonies for purposes of study. Experimentally, this procedure succeeded well enough when the *Micrococcus* was added in considerable quantity to urine, but when the amount inoculated was small, isolation of the *Micrococcus* could not be effected. Trials were then made as to the effect of adding a strong specific serum to these latter growths; it was thought that the serum might cause the aggregation of the *Micrococci* into clumps, and if these were planted out on agar plates a better chance of success might be obtained. The results were encouraging, and in future examinations of the urine of Malta fever cases, it is intended to follow this procedure, as well as the usual dilution method on agar plates.

Experiment I.

An investigation was now undertaken to ascertain whether the *M. melitensis* could live in urine, and especially in a urine which had become alkaline from the decomposition of urea.

A freshly passed urine from a healthy man was inoculated with an emulsion of *M. melitensis* made in distilled water from a recent agar slope. The urine when passed appeared practically sterile. The inoculated urine was placed in a laboratory cupboard and examined daily by plating on nutrose agar. The *Micrococcus* was easily recovered up to and on the 6th day, but could not be detected at a later period. The urine on the 6th day was markedly alkaline from the presence of ammonia, and on titrating it with N/10 acid, the ammonia was found to equal 0.0064 gramme NH_3 per c.c.

This result is of some practical importance as it shows that the *M. melitensis* might be recovered from a urine which had been kept for 6 days and become alkaline in reaction.

The viability of the *M. melitensis* in the presence of ammonia and the comparative absence of saprophytic microbes from the urine in the experiment just related, suggested that, perhaps, this alkali might have a restraining influence on the growth of the bacteria usually found in the urine of Mediterranean fever cases, and so assist in the separation of the specific microbe. Accordingly, broth (+ 10) was treated with pure NH_3 until the amount when titrated with N/10 acid equalled 0.64 gramme per litre. The tubes were incubated and remaining sterile, were then inoculated with *M. melitensis* and with urine from a case of Mediterranean fever. After 24 hours' incubation there was a marked growth of bacteria in the tubes inoculated with urine, but the *M. melitensis* did not show any marked growth until the 4th day. The

result was not unexpected as the work previously done on the reaction of media had shown that the *M. melitensis* did not grow well in alkaline media.

Experiment II.

This experiment was designed in order to ascertain the duration of life of *M. melitensis* when maintained in an absolutely dry state and without a trace of nutrient medium.

A series of sterile cover glasses were placed in a Petri dish and then inoculated with an equal quantity of an emulsion of *M. melitensis*, the cocci from a 48 hours' agar slope being suspended in water. The emulsion was exposed to the air until all traces of moisture had disappeared from the cover glasses. The Petri dish was then placed in a laboratory cupboard, the temperature of which averaged 18° C. Every 24 hours a cover slip was removed and planted out in broth. The resulting growth was plated on agar, and the colonies fished and examined in the following manner:—A likely colony was made into an emulsion with a loopful of broth and then examined under $\frac{1}{12}$ th objective; if cocci were found freely disseminated through the field and showing no signs of clumping, a loopful of serum from an inoculated rabbit was added. When clumping occurred the needle, which had been used to make the emulsion and *not* sterilised, was rubbed over an agar slope. The resulting growth was planted out in glucose peptone, lactose peptone, cane sugar peptone, litmus milk, peptone and salt solution, nitrate broth, and stabbed into gelatine. The growths which resulted corresponded exactly to those obtained when the original *M. melitensis* was planted out in these media.

Result.—A Micrococcus, which corresponded in every particular to the *M. melitensis*, was isolated up to and on the 16th day.

Experiment III.

The object of this experiment was to ascertain the duration of the life of *M. melitensis* in dry soil.

Some soil from a recently manured plot of ground in Gibraltar was powdered, dried, and sterilised, and then inoculated with an aqueous emulsion of *M. melitensis* prepared from an agar slope. The soil was allowed to dry naturally and kept in the laboratory cupboard mentioned in the previous experiment. For a few days traces of moisture were present, but after the 10th day the soil was quite light and formed a black powder which could easily be blown about. The soil was tested weekly for the presence of *M. melitensis*, a portion of the soil being planted out in broth and the resulting growth treated in the manner detailed under Experiment II. Up to and on the 69th day a Micrococcus was recovered, corresponding in every way to the *M. melitensis* originally planted out. During this experiment

careful watch was kept for any change in the morphology of the inoculated microbe. It was thought that the bacillary forms described by Durham might appear, and cause some difficulty in diagnosing the culture. The bacillary forms were never seen, and the *Micrococcus* obtained on an agar slope on the 69th day presented the usual morphology. The cultural characteristics and reaction to the specific serum were also unchanged.

Result.—The *M. melitensis* retained its vitality in dry soil for 69 days.

Experiment IV.

In this experiment a fine sterile sand, practically free from organic matter, was inoculated, and treated in exactly the same manner as the manured soil in Experiment III. The *M. melitensis* was recovered up to and on the 20th day, but not later. The morphology, cultural and serum reactions, were again quite unchanged.

Result.—The *M. melitensis* retained its vitality in dry sand for 20 days.

Experiment V.

The object of this experiment was to discover the duration of life in a foul soil saturated with water. The manured sterile soil employed in Experiment III was inoculated in the same manner as before, but instead of being allowed to dry it was kept saturated with sterile tap-water. The *M. melitensis* was recovered up to and on the 7th day, but could not be detected at a later date, although many trials were made. The result of this experiment seemed to show that immersion in water was inimical to the persistence of the *M. melitensis* in a saprophytic condition.

Result.—The *M. melitensis* retained its vitality in a foul, saturated soil for 7 days.

Experiment VI.

The idea of this experiment was to ascertain the duration of life of the *M. melitensis* when dried on fabrics. Accordingly, pieces of thick regulation blanket, khaki serge, and khaki cotton were inoculated with an emulsion of the microbe made by suspending a recent agar growth in sterile water. The greatest care was taken not to remove any of the nutrient medium. After inoculation the infected fabrics were placed in a Petri dish and allowed to dry naturally; they were then placed in the laboratory cupboard during the whole experiment. Portions of the fabrics were planted out in broth every 3 or 4 days, and the resulting growth plated on nutrient agar in the usual manner. The *M. melitensis* was recovered from the khaki cotton up to and on the 80th day, from the khaki serge on the 80th day, and from the blanket on the 80th day. The morphology, cultural and serum reactions, were again quite unchanged.

Experiment VII.

The rapid disappearance of the *M. melitensis* from the soil saturated with water suggested that an attempt should be made to determine the duration of life of the *M. melitensis* in sterile water. The whole of a recent growth from an agar slope was diffused in 50 c.c. of sterile tap-water, representing an exceedingly gross pollution. The flask was kept in the laboratory cupboard, and every day 1 c.c. was plated on nutrose agar. The Micrococcus was readily isolated for 6 days, but on the 7th and 13th days it could not be detected.

Experiment VIII.

This experiment was a repetition of Experiment VII, but instead of planting out small quantities from day to day, the flask was left undisturbed for 3 weeks. Broth was then added so as to enrich the whole bulk of the water, and the flask incubated at 37° C. for 3 days. The growth which resulted was found to contain numerous small cocci decolorised by Gram's method. A portion of the growth was then added to an equal quantity of a strong rabbit serum diluted 1—10, and the whole thoroughly mixed was drawn up into a capillary pipette. Distinct agglutination having occurred, the pipette was then opened and the agglutinated mass stroked over a series of agar plates; unfortunately a pure culture of the *M. melitensis* was not obtained. The result of this experiment is not conclusive, but it suggests that the duration of life of the *M. melitensis* in water may be longer than 1 week.

Conclusions.

(1) The *M. melitensis* is able to live for 6 days in a urine which has become alkaline from the presence of ammonia.

(2) The *M. melitensis* survives for 16 days when spread in a thin layer on a glass cover slip.

(3) The *M. melitensis* survives for 69 days when planted in a dry sterilised manured soil.

(4) In dry sterilised sand the duration of life of the *M. melitensis* appears to be only 20 days.

(5) In a sterilised manured soil saturated with water the *M. melitensis* appears to survive for only 7 days.

(6) The *M. melitensis* is able to live for 80 days on dry fabrics, such as blanket, khaki serge, and khaki cotton.

(7) The *M. melitensis* appears to live for a comparatively short time in sterilised tap-water. It was only recovered in pure culture 6 days after being planted out, though from the result of Experiment VIII it appears possible that the duration of life may extend to 3 weeks.

2.

FURTHER STUDIES ON THE SAPROPHYTIC EXISTENCE OF *MICROCOCCUS MELITENSIS*.

By Major W. H. HORROCKS, R.A.M.C., Member Mediterranean
Fever Commission.

(Received September 17, 1904.)

1. DURATION OF LIFE OF THE *M. melitensis* IN STERILISED TAP- WATER.

Experiment I.

In the Gibraltar report it was stated that the duration of life of the *M. melitensis* in sterilised tap-water was probably longer than the recorded experiments indicated. Accordingly, the experiment of adding an emulsion of *M. melitensis*, made by carefully mixing the growth from an agar slope in sterile water to a known volume of water, was repeated. In this case 1 c.c. of an emulsion made from a strain of *M. melitensis* isolated from urine was added to 10 c.c. of sterilised tap-water. Chemical analysis showed that the tap-water was very pure, and contained practically no organic material. The emulsion was added to the tap-water on August 1, 1904, and at various times 0.5 c.c. was removed, and added to 10 c.c. of broth, the contents of the tube being thoroughly mixed and then incubated at 37° C. As soon as the broth tube showed any signs of growth a large loopful was stroked in a zig-zag manner over an agar slope, which was then incubated at 37° C. On August 15, 1904, a pure culture of *M. melitensis* was isolated, the growth responded to all the usual cultural tests, and agglutinated at once with the serum of Monkey 45, diluted 1—1000. On August 21, 1904, the same procedure was followed, and the *M. melitensis* again isolated. On August 27, 1904, a pure culture of *M. melitensis* was obtained, and appeared quite unchanged. On September 6, 1904, the specific microbe was again isolated.

Result.—The *M. melitensis*, derived from urine, appears to survive for 37 days in sterilised tap-water.

2. DURATION OF LIFE OF THE *M. melitensis* WHEN PLANTED OUT IN SOIL.

In the Gibraltar experiments already recorded a manured garden soil and a dry sand were employed. Valletta and Sliema are mainly built on the Globigerina limestone, and the white dust which abounds on the roads is chiefly due to the attrition of this stone; occasionally the soil has a red colour, due to the presence of oxide of iron resulting from the oxidation of FeS_2 (iron pyrites).

Experiment II.

A grey coloured soil was obtained from Sliema, and ground into a fine powder. According to Sir John Murray's analysis, this soil has the following composition:—

Carbonate of lime, iron, and alumina ($\text{CaCO}_3, \text{Fe}_2\text{O}_3, \text{Al}_2\text{O}_3$)	78.39
Phosphate of lime (Ca_3PO_4)	2.70
Magnesium carbonate (MgCO_3)	0.44
Calcium sulphate (CaSO_4)	0.33
Insoluble in dilute HCl (1—10)	17.87
	<hr/>
	99.73

The soil was carefully dried and sterilised, and a portion planted out in broth and incubated at 37°C . After 4 days' incubation there were no signs of growth, showing that sterilisation had been effected. On July 15, 1904, the soil was inoculated with an emulsion of *M. melitensis*, made by suspending the growth on an agar slope in distilled water, and allowed to dry naturally. On July 23, 1904, a portion of the soil, still showing faint traces of moisture, was planted out in broth and incubated at 37°C . On July 26, 1904, a growth occurred in the broth culture, which was planted out on an agar slope; two days later a typical growth, which responded to all the characteristic tests, appeared. On July 30, 1904, the soil was noted to be practically dry. On August 11, 1904, a portion of the soil was removed and treated in the same manner as on July 23, 1904; a typical growth of the *M. melitensis* was again obtained. On August 19, 1904, the same procedure was followed, and a pure culture of the specific microbe was isolated. On August 27, 1904, the *M. melitensis* was again isolated.

Result.—The *M. melitensis* survived for 43 days in a soil, which was allowed to dry naturally, and which was free from appreciable traces of moisture for 27 days.

Experiment III.

In this experiment a reddish coloured soil, also obtained from Sliema, was employed. Sir John Murray's analysis of this soil gave the following results:—

Carbonate of lime (CaCO_3).....	80.24
Phosphate of lime ($\text{Ca}_3\text{2PO}_4$).....	3.57
Magnesium carbonate (MgCO_3).....	1.63
Calcium sulphate (CaSO_4).....	0.06
Iron and alumina (Fe_2O_3 and Al_2O_3).....	1.13
Insoluble in dilute HCl (1 in 10)	12.88
	<hr/> 99.51

The soil was sterilised, and its sterility tested as in Experiment I. On June 25, 1904, it was inoculated with an emulsion of *M. melitensis*, made in sterile water from an agar slope grown for 48 hours at 37° C. The soil, having been dried in the incubator at 37° C., was placed in the laboratory cupboard. On July 4, 1904, a portion of the soil was planted out in broth, and the growth which resulted on July 7, 1904, was planted out on an agar slope. A typical culture, giving all the reactions of the *M. melitensis*, was obtained.

On July 11, 1904, the soil was again tested, and a pure culture of *M. melitensis* was isolated.

On July 15, 1904, an examination was made, but the growth in broth did not take place for 9 days, showing that the organism was much enfeebled. On planting out the growth on agar only a few colonies of the *M. melitensis* were obtained. On July 24, 1904, and on July 30, 1904, examinations were made, but the results were negative, the *M. melitensis* having apparently died out.

Result.—The *M. melitensis* lived for 21 days in red Sliema soil, thoroughly dried immediately after inoculation.

Experiments IV and V.

These experiments were designed in order to ascertain whether the presence of traces of moisture, as distinguished from flooding of the soil, had any influence on the survival of the *M. melitensis*.

In Experiment IV white Globigerina limestone dust was inoculated with *M. melitensis* on July 8, 1904; the tube was then placed in the laboratory cupboard. About once a week a little sterile tap-water was added by means of a pipette, so as to preserve a faint appearance of moisture on the surface of the soil. At various intervals portions of the soil were removed and planted out in broth, the tube being then incubated at 37° C. The resulting growth was planted on agar and tested as already described under Experiment I.

The *Micrococcus melitensis* was isolated on July 15, 1904.

"	"	"	July 24, 1904.
"	"	"	July 30, 1904.
"	"	"	August 11, 1904.
"	"	"	August 19, 1904.

The Micrococcus melitensis was isolated on August 27, 1904.

" " " September 7, 1904.

" " " September 19, 1904.

Result.—The *M. melitensis* survived for 72 days in a damp soil.

In Experiment V the red soil, described under Experiment II, was employed. The soil was inoculated on July 8, 1904, and the testings carried out at the same time as in Experiment III. The *M. melitensis* was isolated after 72 days' immersion in this soil.

3. SURVIVAL OF THE *M. melitensis* AFTER EXPOSURE TO THE SUN.

Experiment VI.—Exposure on Thin Strips of Glass.

A 36-hours' growth of *M. melitensis* on nutrose agar was made into an emulsion with sterile tap-water. A series of thin glass cover slips were sterilised and the surface of each inoculated with the emulsion by means of a sterile pipette. The cover slips were then exposed to the sun as follows :—

On June 17, 1904, from 9.30 A.M. to 11 A.M. Maximum temperature in the sun, 130° F. (54°·4 C.).

On June 17, 1904, from 3.10 P.M. to 4.10 P.M. Maximum temperature in the sun, 130° F. (54°·4 C.).

On June 19, 1904, from 10.15 A.M. to 12.15 P.M. Maximum temperature in the sun, 133° F. (56°·1 C.).

After each exposure one of the cover slips was added to sterile broth and incubated at 37° C. The broth tubes all remained sterile, though the incubation was maintained for 14 days.

From control slips, not exposed to the sun, the *M. melitensis* was easily recovered.

Experiment VII.—Exposure in a Very Thin Layer of Soil.

Samples of white and red soils, already mentioned under the soil experiments, were spread in layers, $\frac{1}{8}$ inch deep, on the bottom of glass dishes, and then inoculated with an emulsion of *M. melitensis*, made from an agar slope as mentioned above. The dishes were exposed to the sun as follows :—

On June 20, 1904, from 12.15 P.M. to 1 P.M. Maximum temperature in the sun, 128° F. (53°·3 C.).

On June 21, 1904, from 8.50 A.M. to 11.50 A.M. Maximum temperature in the sun, 135° F. (57°·2 C.).

On June 22, 1904, from 8.45 A.M. to 11.45 A.M. Maximum temperature in the sun, 126° F. (52°·2 C.).

On July 1, 1904, from 10.30 A.M. to 12.30 P.M. Maximum temperature in the sun, 133° F. (56°·1 C.).

After each experiment particles from the dried baked surface were planted out in broth, and any resulting growth was then planted out on agar and the growth tested for agglutination, etc. The *M. melitensis* was recovered after the exposure on June 21, 1904, representing $3\frac{1}{2}$ hours' exposure to direct sunlight, but not later.

The *M. melitensis* was readily obtained from a control soil after 21 days in the laboratory cupboard.

Experiment VIII.—Exposure on Khaki Drill.

A piece of khaki drill was inoculated with the same emulsion used in the previous experiments. The drill was then exposed to the sun as follows:—

On June 17, 1904, from 9.30 A.M. to 11 A.M. Maximum temperature in the sun, 130° F. ($54^{\circ}\cdot4$ C.).

On June 17, 1904, from 3.10 P.M. to 4.10 P.M. Maximum temperature in the sun 130° F. ($54^{\circ}\cdot4$ C.).

On June 19, 1904, from 10.15 A.M. to 12.15 P.M. Maximum temperature in the sun, 133° F. ($56^{\circ}\cdot1$ C.).

After each exposure a portion of the infected drill was cut off and planted out in broth, and the resulting growth planted out on agar and tested in the usual manner.

The *M. melitensis* was recovered after an exposure of not more than $2\frac{1}{2}$ hours to the sun.

Experiment IX.—Exposure on Soil $\frac{1}{2}$ -inch Deep.

The idea of this experiment was to ascertain whether the deeper layers of the soil, which were quite dry and capable of being blown about by strong winds, would still retain infection after exposure to the sun.

The white Globigerina limestone soil, previously described, was sterilised and carefully poured into a sterile Petri dish so as to form a uniform layer $\frac{1}{2}$ inch deep. The soil was then inoculated with an emulsion of *M. melitensis*, made by suspending the growth on an agar slope, inoculated from a urine culture and incubated for 48 hours at 37° C. The soil was exposed to the sun as follows:—

August 19, 1904, 3.30 P.M. to 4.30 P.M. Maximum temperature in the sun, 147° F. ($63^{\circ}\cdot8$ C.).

August 20, 1904, 9 A.M. to 11.45 A.M. Maximum temperature in the sun, 153° F. ($67^{\circ}\cdot2$ C.). After the total exposure of $3\frac{1}{2}$ hours, a portion from the surface was planted out in broth, so as to compare this experiment with the one previously reported.

August 21, 1904, exposed from 9.30 A.M. to 11.30 A.M. Maximum temperature in sun, 154° F. ($67^{\circ}\cdot7$ C.). After a total exposure of

5½ hours, portions of soil taken from the surface and from the depth were planted out in broth tubes.

August 22, 1904, exposed from 9 A.M. to 11.15 A.M. Maximum temperature in sun 148° F. (64°·4 C.). Portions of soil from the surface and depth again planted out in broth.

August 23, 1904, exposed from 10.15 A.M. to 11.15 A.M. Maximum temperature in the sun, 148° F. (64°·4 C.). Planted out portions of soil from the surface and depth in broth tubes.

August 25, 1904, exposed from 10.15 A.M. to 11.15 A.M. Maximum temperature in the sun, 146° F. (63°·3 C.). Total exposure since the 19th equals 10 hours. Planted out portions of soil from the surface and depth in broth tubes.

September 6, 1904. All the broth tubes which had been incubated at 37° C., since the date of inoculation, were planted out on agar slopes.

September 12, 1904. All the agar tubes inoculated with the broth containing the surface soil, have remained quite sterile.

September 12, 1904. The agar tubes inoculated with the broth containing the portions of soil taken from the depth after 5½ and 8 hours' exposure, show a growth of *B. mesentericus*. There is no sign of the *M. melitensis*.

The agar tubes inoculated with the broth tubes containing the soil from the depth after 9 and 10 hours' exposure are quite sterile.

Result.—The heat derived from exposure to the sun, the maximum temperature varying between 146° F. and 153° F., apparently destroys the *M. melitensis* at a depth of ½ inch from the surface.

Experiment X.—Duration of Life of the M. melitensis when Planted out in Sea-Water.

Sea-water was obtained from the harbour and sterilised. A portion was then planted out on agar and in broth; both the tubes remained sterile after incubation at 37° C.

On July 25, 1904, a tube containing 10 c.c. of sterile sea-water was inoculated with the growth obtained from an agar slope, incubated for 13 days at 37° C. The inoculated tube was placed in the laboratory cupboard. On July 29, 1904, 0·5 c.c. was removed from the tube and planted out in broth; on September 2, 1904, there was a distinct growth in the broth; the growth was planted out on an agar slope, and a typical growth of *M. melitensis* was obtained, which responded to the classical tests.

On July 31, 1904, 0·5 c.c. was planted out in broth, and the same procedure followed as on July 29, 1904; a typical growth of *M. melitensis* was obtained.

On August 5, 1904, 0·5 c.c. was planted out in broth; a growth of *M. melitensis* resulted.

On August 8, 1904, 0.5 c.c. was planted out as before, and a pure culture of *M. melitensis* was obtained.

On August 12, 1904, 0.5 c.c. was planted out in broth ; the resulting growth when planted on an agar slope gave rise to a growth, which agglutinated very slowly with the serum from Monkey 45. A portion of the growth was planted out in glucose and litmus milk ; the glucose was not fermented, and the litmus milk became alkaline, without showing the slightest trace of coagulation or digestion. The growth also had a typical morphology, and did not stain by Gram's method.

On August 15, 1904, 0.5 c.c. was planted out in broth, and a culture again obtained, which was typical of *M. melitensis*, except that the agglutination occurred slowly.

On August 19, 1904, 0.5 c.c. was planted out, and the same result obtained as on August 12 and 15, 1904. The growth was tested with the specific serum which, diluted 1—1000, caused instantaneous agglutination of the laboratory standard culture of *M. melitensis*. With the growth from sea-water, this serum, diluted 1—1000, caused clumping in $\frac{1}{2}$ hour.

On August 22, 1904, 0.5 c.c. was planted out in broth, and incubated at 37° C. No sign of growth appeared after 15 days' incubation.

On August 26, 1904, 0.5 c.c. was again planted out, but no growth appeared.

Result.—The *M. melitensis* appears to survive for 25 days in sterilised sea-water.

Conclusions.—1. The *M. melitensis* retains its vitality in sterilised tap-water for 37 days.

2. In a Maltese soil, allowed to dry naturally, the *M. melitensis* survives for 43 days ; and in one thoroughly dried immediately after inoculation, it survives for 21 days.

3. The *M. melitensis* survives for 72 days in a damp soil.

4. Exposure to the sun for a few hours kills the *M. melitensis*.

5. The *M. melitensis* survives for 25 days in sterilised sea-water.

ON THE RECOVERY OF THE *MICROCOCCUS MELITENSIS* FROM THE URINE, FÆCES, AND SWEAT OF PATIENTS SUFFERING FROM MEDITERRANEAN FEVER.

By Major W. H. HORROCKS, R.A.M.C., Member Mediterranean
Fever Commission.

(Received September 17, 1904.)

Note.—The work on the examination of urine, fæces and mosquitoes has been done
in conjunction with Captain Kennedy, R.A.M.C.

1. *Examination of Urine.*

In my report on previous work performed at Gibraltar, it was pointed out that the ordinary restraining agents, such as carbolic acid, sodium taurocholate, malachite green, etc., could not be depended upon to inhibit the growth of the micro-organisms usually found associated with the *M. melitensis* in the urine of Mediterranean fever cases. Accordingly, in the earlier work at Malta, attempts were made to isolate the Micrococcus by first enriching a known bulk of urine with broth, usually in the proportions of 1—1 and 1—3, and then, after varying periods of incubation at 37° C., plating the growths, which resulted, on nutrose agar. It was hoped that, under these conditions, the specific microbe would so multiply as to enable colonies to be detected by the plate method. A very short experience showed that the enrichment method was not satisfactory; the extraneous organisms multiplied more vigorously than the *M. melitensis*, and the latter was completely crowded out. It was then decided to make use of the glucose-litmus-nutrose-agar plates, already mentioned in the Gibraltar report, and to add small quantities of urine, 0.25—0.33 c.c., to these plates, allowing the urine to flow over and form a thin layer on the surface of the solidified agar. This procedure enabled the actual number of colonies of the Micrococcus passed in the urine to be ascertained. Before collecting the urine for investigation, the genitalia were washed with carbolic acid lotion; the patient then passed urine, but the first portion, which acted as a flush to the urethra, was discarded. On the glucose-litmus-nutrose-agar plates, the colonies of the *M. melitensis* appeared as almost transparent deep blue drops; likely colonies were next fished, and made into an

emulsion with normal salt solution on a cover-glass. It may be noted that the *M. melitensis* readily emulsifies, and the culture appears to flow off the point of the needle into the surrounding fluid; this characteristic was found of great assistance in detecting the specific microbe. A streptococcus is found in urine which produces, on the special plates, colonies very closely resembling those of the *M. melitensis*; when fished, however, they do not readily emulsify, and, on examination, under one-twelfth, are found to consist of a medium-sized coccus, staining with Gram. When it was found that the colony readily emulsified, the hanging drop was carefully examined under the oil immersion, in order to ascertain the nature of the organism, and to make sure that no false clumps were present. If the microbe presented the characteristics of the *M. melitensis*, and the emulsion was satisfactory, the cover-glass was removed, and a little specific animal serum added. In the earlier work I employed a rabbit serum prepared at Gibraltar, but, in the later work, serum from Monkey 45 was used. When the microbe under examination manifested instantaneous clumping under the influence of the serum, a portion of the colony was planted out on an agar slope, and incubated at 37° C. The resulting growth was then treated as follows:—

(1) Tested with the serum of Monkey 45. This serum, when diluted 1—1000, was found to cause instantaneous clumping, visible to the naked eye, of the laboratory stock culture of *M. melitensis*.

(2) Planted in glucose-litmus-peptone, or on a glucose-litmus-agar slope, and incubated for 7 days at 37° C.

(3) Planted in litmus milk and incubated for a month at 37° C.

(4) Examined as to retention of stain by Gram's method.

A micro-organism, which agglutinates with a specific animal serum in a high dilution, does not ferment glucose, renders milk alkaline without coagulation, may justly be regarded as the *M. melitensis*.

All the strains of *M. melitensis*, which have been isolated from the urine of Mediterranean fever cases, have responded to these tests.

Employing the above technique the first successful isolation was obtained from the urine of Sergeant Pudney, 2nd Essex Regiment. A plate made with 0.33 c.c. of urine was found to contain thirty-three colonies, after 5 days' incubation at 37° C.; colonies were first observed on the 4th day, but the maximum number did not appear until the 5th day of incubation.

The *M. melitensis* has now been isolated thirty-nine times, and from the urine of thirteen different patients. Colonies were never observed before the 3rd day of incubation, and at this period they were usually very minute and easily missed; on the 4th day of incubation, however, they were readily detected on the glucose-litmus-nutrose-agar plates. The actual numbers of *M. melitensis* isolated from urine are shown in the attached table (A).

Table A.—Showing the Number of Colonies of *M. melitensis* found in each Sample of Urine.

Name.	Date.	Quantity of urine in c.c.	No. of colonies in each plate.	No. of colonies per c.c.	Average No. per c.c.
Howe	6.7.04	Isolated from broth culture made with urine obtained at the <i>post-mortem</i> examination.			
Pudney	18.7.04	0.5	1	2	
"	25.7.04	0.33	33	99	50
		0.25	3		
		0.25	3	12	26
		0.25	3		
		0.25	21	84	
		(mucus)			
"	27.7.04	0.33	2	6	6
Martin	2.8.04	0.33	95	285	285
Markham	6.8.04	0.25	5	20	20
Breuster	6.8.04	0.25	2	8	8
Belfield	6.8.04	0.33	1	3	3
Pudney	7.8.04	0.125	1	8	8
"	8.8.04	0.5	4	8	8
Fisher	8.8.04	0.33	3	9	15
		0.33	7	21	
Lawson	12.8.04	0.25	1	4	4
Breuster	13.8.04	0.25	2	8	8
Lawson	14.8.04	0.33	4	12	12
"	14.8.04	0.33	5	15	15
"	15.8.04	0.25	6	24	24
Lawrence	16.8.04	0.25	1	4	4
		0.25	1	4	
Lawson	17.8.04	0.25	45	180	180
Fisher	19.8.04	0.25	1	4	4
Lawson	20.8.04	0.33	12	36	36
Griffin	20.8.04	0.25	10	40	40
Lawson	21.8.04	0.33	15	45	45
Griffin	23.8.04	0.25	1	4	4
Lawson	23.8.04	0.33	105	315	315
Pudney	23.8.04	0.33	2	6	6
Breuster	24.8.04	0.25	1	4	4
Griffin	24.8.04	0.25	1	4	4
Lawson	26.8.04	0.33	1	3	3
Markham	26.8.04	0.33	1	3	3
Lawson	29.8.04	0.33	3	9	9
Barry	29.8.04	0.33	1	3	3
Christie	29.8.04	0.33	4	12	12
Lawson	31.8.04	0.33	1	3	3
Lawrence	1.9.04	0.33	3	9	9
Markham	1.9.04	0.33	1	3	3
Griffin	9.9.04	0.33	5	15	15
Lawrence	10.9.04	0.33	70	210	210
Kinsella	16.9.04	0.33	1	3	15
		0.33	9	27	
Markham	28.9.04	0.5	298	596	596

Up to the present time the *Micrococcus* has not been isolated from urine earlier than the 15th day or later than the 82nd day of disease.

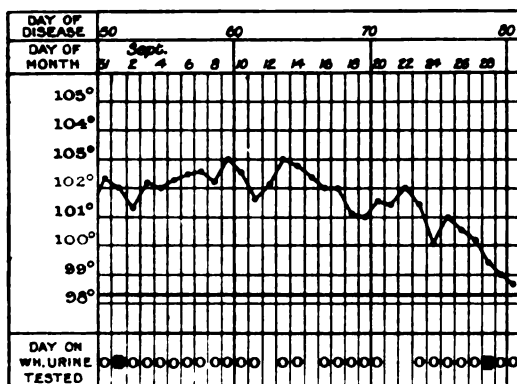
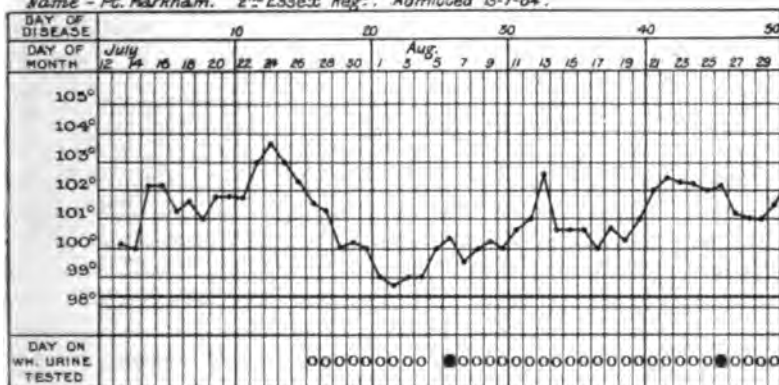
It is present in the urine of patients who are sufficiently convalescent to be allowed up, but still have an evening rise of temperature.

In order to save repetition and to enable the work done to be grasped at a glance, the attached charts have been prepared by Captain Kennedy, Royal Army Medical Corps, who has given me most valuable assistance throughout the work. Each square represents a day of disease, and in every case the chart commences with the day which, after careful questioning of the patient, was considered to be probably the 1st day of disease; so that on looking through the charts the different columns represent the same day of disease for each patient. The course of the fever is represented by the evening temperature, and the 0 sign indicates an examination made without any result; the Maltese cross sign represents a successful isolation of the *M. melitensis*. It will be noticed that there are many failures as compared with successes. In the earlier work the constant want of success was undoubtedly due to the faulty method of procedure; but in the later work it is to be attributed partly to the fact that the *M. melitensis* is not voided in the urine every day, but appears in gushes at uncertain periods, and partly to the presence in the urine of acid-producing organisms, which out-grow and interfere with the development of the *M. melitensis*.

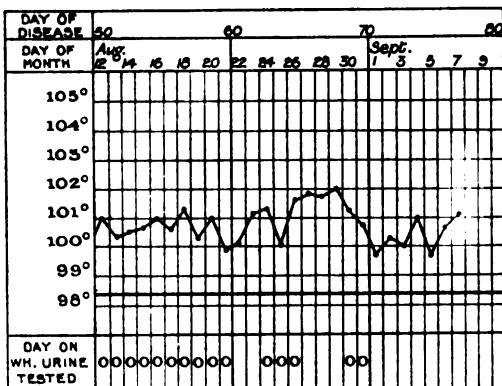
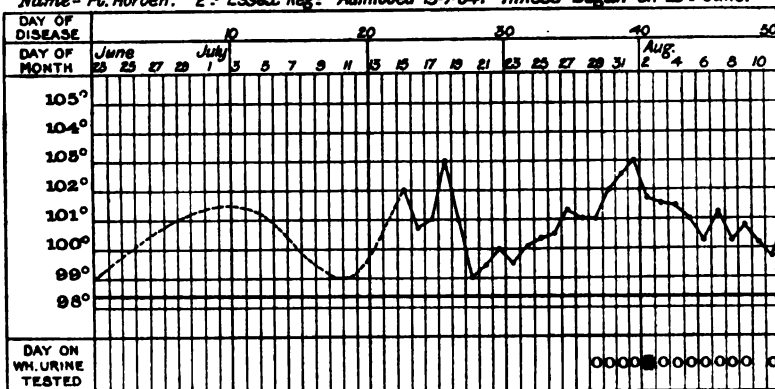
Careful observation of the urines has been made in order to ascertain whether any physical or chemical change is associated with the passage of the *M. melitensis*. All the urines have been free from the general opacity or turbidity, which is associated with Typhoid Bacilluria. A little deposit of mucus has been observed, and a portion of this when plated out has always given more colonies than the clear portion of the urine treated in the same manner. On three occasions a trace of albumen was noticed, but up to the present no physical or chemical change common to all the urines and indicating the passage of the *M. melitensis* has been observed.

Table A shows the number of micrococci per cubic centimetre obtained from each sample of urine, and indicates the dates when the isolation was effected. It will be noticed that the numbers of micrococci excreted are small as compared with the figures recorded by several observers during the bacilluria of typhoid fever. It is possible that the figures given in the table do not represent the actual numbers passed in every case, and that many colonies escaped observation owing to their being crowded out by other microbes. At the same time many of the plates, notably those of Sergeant Pudney and Private Lawson, were nearly pure cultures of *M. melitensis*, and as all the colonies which appeared were perfectly discrete, and there was ample room in the plates for other colonies to develop had they been present, it does not seem probable that the numbers passed greatly exceeded the maximum figures recorded.

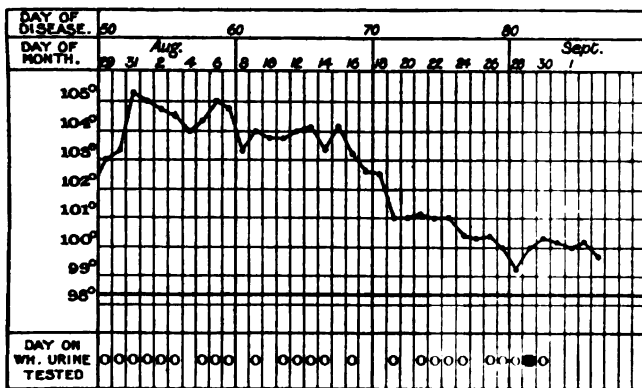
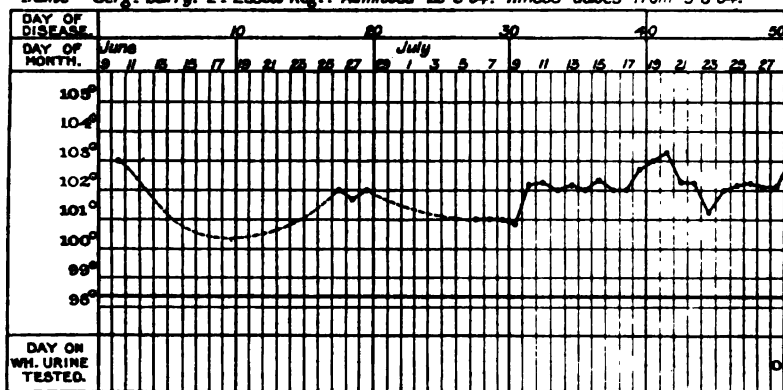
Name - P. Markham. 2nd Essex Reg^t. Admitted 15-7-04.

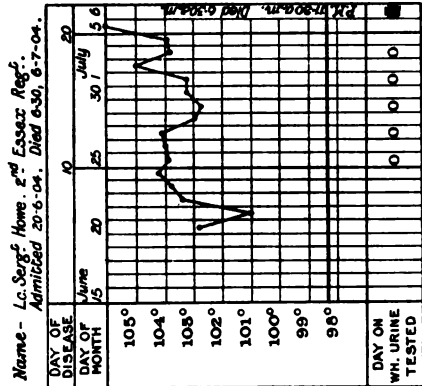


Name- *Pt. Morten. 2nd Essex Regt Admitted 15-7-04. Illness began on 25th June.*

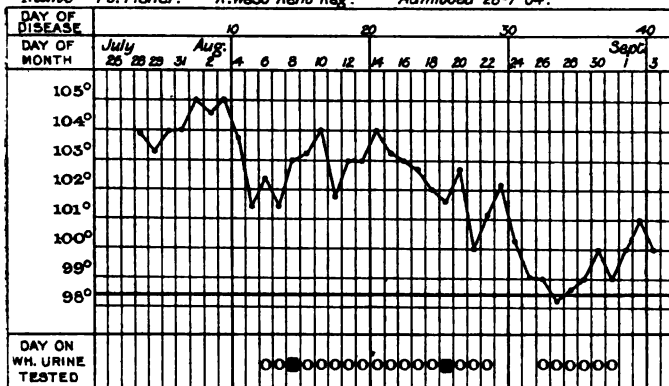


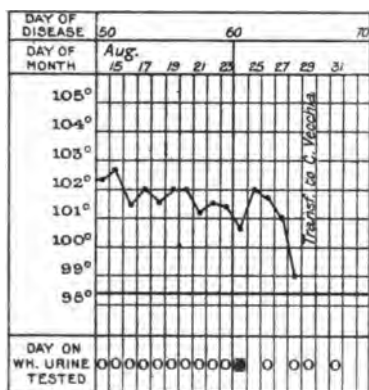
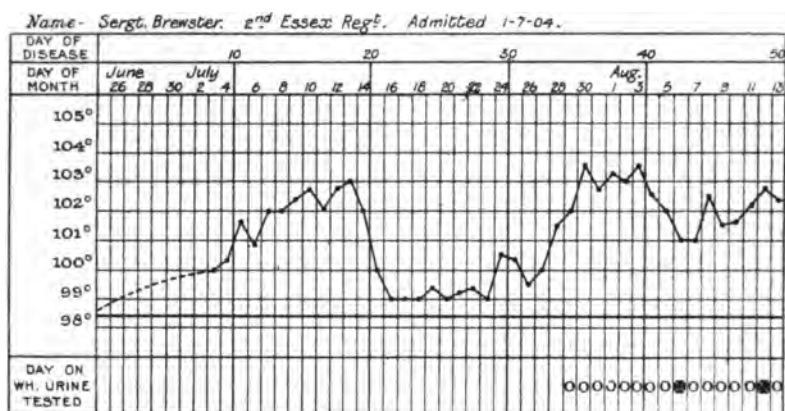
Name - Serg^t Barry. 2^d Essex Reg^t. Admitted 26-6-04. Illness dates from 9-6-04.





Name - *Pt. Fisher.* R. West Kent Reg^t Admitted 26-7-04.





Up to the present 520 samples of urine have been examined, representing the study of more than 1000 plates.

2. Examination of Fæces.

Having succeeded in isolating the *M. melitensis* from the urine of Mediterranean fever cases, attempts were now made to detect the microbe in the fæces of these patients. Unfortunately, most of the cases suffered from constipation, and the bowels only acted after the administration of an enema. A few patients suffered from diarrhoea for a short time, and the opportunity was taken of investigating these stools.

The great difficulty to contend with in the study of fæces is caused by the presence of the rapidly growing *B. coli* in large numbers. The enrichment method, which failed with the urine, appeared to be even less likely to yield satisfactory results with fæces. A few trials were made of planting out some of the stools in broth and then, after incubating for four days at 37° C., plating out the growths on glucose-litmus-nutrose-agar plates. The results were highly unsatisfactory; the *B. coli* and its allies converted the plates into a strongly acid medium, on which the *M. melitensis* would not grow. Evidently a medium on which the *B. coli* could not develop, would prove of great assistance in isolating the *M. melitensis* from stools. E. Roth in the *Archiv f. Hygiene* of March 3, 1904, reported that the development of the *B. coli* was arrested in a medium containing 60 per cent. of a solution containing $\frac{1}{100}$ th of caffeine. For greater security against the development of *B. coli* he recommended the proportion of caffeine to be increased to 115 per cent. of the $\frac{1}{100}$ th solution. Ficker and Hoffmann in the same number of the *Archiv f. Hygiene* also attested the value of caffeine in arresting the development of *B. coli*; they used 5 grammes of caffeine per litre of fluid. Courmont and Lacomme also wrote on caffeine in bacteriology in the March number of the *Journal of Physiology and Pathology*, 1904. They stated that when caffeine was added to broth to the extent of 1 per cent., the development of *B. coli* was prevented. In view of these statements, experiments were made to test the viability of the *M. melitensis* in caffeinated media. Broth tubes were prepared containing 0.5 per cent., 0.75 per cent., and 1 per cent. of caffeine. Each tube was inoculated with a small loopful of an agar growth, derived from the spleen of Sergeant Howe. The results obtained were as follows:—

- (1). 5.8.04, 0.5 per cent. Caffeine broth, inoculated with *M. melitensis* spleen culture of man, incubated at 37° C.
- (2). 5.8.04, 0.75 per cent. Caffeine broth, inoculated with *M. melitensis* spleen culture of man, incubated at 37° C.
- (3). 5.8.04, 1 per cent. Caffeine broth, inoculated with *M. melitensis* spleen culture of man, incubated at 37° C.

(1). 8.8.04, 0.5 per cent. Good growth. Planted out on agar and *M. melitensis* recovered.

(2). 8.8.04, 0.75 per cent. No growth.

(3). 8.8.04, 1 " "

(2). 9.8.04, 0.75 " "

(3). 9.8.04, 1 " "

(2). 10.8.04, 0.75 " "

(3). 10.8.04, 1 " "

(2). 11.8.04, 0.75 " "

(3). 11.8.04, 1 " "

(2). 12.8.04, 0.75 " "

(3). 12.8.04, 1 " "

(2). 15.8.04, 0.75 " "

(3). 15.8.04, 1 " "

(2). 18.8.04, 0.75 per cent. No growth. Planted out on agar slopes. No growths appeared.

(3). 18.8.04, 1 per cent. No growth. Planted out on agar slopes. No growths appeared.

Result.—*M. melitensis* derived from the spleen of man does not appear to develop in media containing more than 0.5 per cent. of caffeine.

Courmont and Lacomme having stated in their paper that cultures of *B. typhosus* from urine were more resistant to the action of caffeine than cultures derived from the blood, experiments were made to see if the same held good for cultures of *M. melitensis*. Accordingly, batches of the same broth used in the previous experiments were inoculated with an agar culture obtained from Sergeant Pudney's urine; the tubes were incubated at 37° C.

The results obtained were as follows :—

(1). 5.8.04, 0.5 per cent. Caffeine broth, inoculated with culture from urine.

(2). 5.8.04, 0.75 per cent. Caffeine broth, inoculated with culture from urine.

(3). 5.8.04, 1 per cent. Caffeine broth, inoculated with culture from urine.

(1). 8.8.04, 0.5 per cent. Good growth. Planted on agar. *M. melitensis* recovered.

(2). 8.8.04, 0.75 per cent. Very feeble growth. Planted on agar. *M. melitensis* recovered.

(3). 8.8.04, 1 per cent. Very feeble growth. Planted on agar. *M. melitensis* recovered.

Result.—The *M. melitensis* derived from urine is able to grow, but only feebly, in broth containing 0.75 and 1 per cent. of caffeine.

A culture of *B. coli* isolated from the stool of a Mediterranean fever case was next tested as to its growth in caffeinised broth. The results obtained were as follows :—

(1). 16.8.04, 0.5 per cent. Caffeine broth, inoculated with *B. coli* from stool of Mediterranean fever case.

(2). 16.8.04, 0.75 per cent. Caffeine broth inoculated with *B. coli* from stool of Mediterranean fever case.

(3). 16.8.04, 1 per cent. Caffeine broth, inoculated with *B. coli* from stool of Mediterranean fever case.

(1). 17.8.04, 0.5 per cent. Good growth. Planted on agar. *B. coli* recovered.

(2). 17.8.04, 0.75 per cent. No growth.

(3). 17.8.04, 1 " "

(2). 18.8.04, 0.75 " "

(3). 18.8.04, 1 " "

(2). 19.8.04, 0.75 per cent. Feeble growth. Planted on agar. A few colonies of *B. coli* appeared.

(3). 19.8.04, 1 per cent. Feeble growth. Planted on agar. A few colonies of *B. coli* appeared.

Result.—Caffeine in the proportion of 0.75 and 1 per cent. appeared to have a distinct restraining influence on the growth of *B. coli*.

An emulsion of one loop of *B. coli* and one loop of *M. melitensis*, from a urine culture, was now thoroughly mixed and then plated out on 0.75 per cent. caffeine-glucose-nutrose-litmus-agar. As a result a few colonies of *B. coli* appeared in 48 hours, but no signs of the *M. melitensis* were observed even after 6 days' incubation at 37° C.; evidently the use of media containing more than 0.50 per cent. of caffeine would be attended with considerable risk of arresting the growth of the *M. melitensis*.

A batch of plates, containing 0.5 per cent. of caffeine in addition to the usual glucose-nutrose-litmus-agar, was now prepared. An emulsion of a stool from a Mediterranean fever case was plated out, and as a control the same emulsion in the same quantities was plated on the ordinary glucose-nutrose-litmus-agar. After 48 hours' incubation at 37° C., there was no appreciable difference between the plates, so the use of caffeine was abandoned in this investigation. The technique has consisted in adding loopfuls of the fluid stools, the number of loops depending on the fluidity of each stool, to either sterile salt solution or broth until a slightly opalescent mixture was produced. Loopfuls of the mixture were then stroked concentrically or diffused by means of a "platinum spreader" over the surface of glucose-litmus-nutrose-agar, solidified in Petri dishes. The plates were then placed with the covers downwards in the 37° C. incubator. After 4 and 5 days' incubation the resulting colonies were examined in a hanging drop; if anything like the morphology of *M. melitensis* appeared, the cover-glass was removed, and a loopful of the specific serum, diluted 1—10, added. Many of the streptococci occurring in stools bear a superficial resemblance to the *M. melitensis*; still, as a rule,

Table showing the Days of Disease on which the Stools were Examined, and the Number of Plates made.

[illegible]

the colonies have a faint opacity and sometimes a reddish tinge which enables them to be at once distinguished from the *M. melitensis*. In any case of doubt the addition of the specific serum enabled a diagnosis to be made. The attached table shows the number of stools examined and the results up to the present time. It will be seen that 1026 plates made from eighty-six stools have been studied, but with a negative result.

Examination of Stools of Mediterranean Fever Cases.

Name.	Dates.	Number of plates.	Day of disease.	Result.
1. Barry	31.7.04	6	53	<i>M. melitensis</i> not isolated.
2. "	23.8.04	12	76	" "
3. "	24.8.04	4	77	" "
4. Eldred	27.7.04	10	27	" "
5. "	26.7.04	4	28	" "
6. Francis	17.7.04	3	19	" "
7. "	18.7.04	3	20	" "
8. Vince	23.7.04	5	18	" "
9. "	17.8.04	9	43	" "
10. "	24.8.04	4	50	" "
11. Moore	25.7.04	5	25	" "
12. Brewster	5.8.04	5	42	" "
13. Jones	7.8.04	4	55	" "
14. "	8.8.04	3	56	" "
15. "	9.8.04	4	57	" "
16. Griffin	11.8.04	4	15	" "
17. "	15.8.04	8	19	" "
18. "	16.8.04	9	20	" "
19. "	17.8.04	4	21	" "
20. "	19.8.04	4	23	" "
21. "	21.8.04	21	25	" "
22. "	23.8.04	4	27	" "
23. Mays	12.8.04	4	40	" "
24. Fisher	14.8.04	8	21	" "
25. "	15.8.04	19	22	" "
26. "	16.8.04	3	23	" "
27. "	17.8.04	6	24	" "
28. "	18.8.04	16	25	" "
29. "	19.8.04	8	26	" "
30. Christie	2.9.04	21	23	" "
31. Lawrence	2.9.04	8	62	" "
32. Hurrell	23.8.04	24	23	" "
33. Fisher	23.8.04	16	30	" "
34. Hurrell	25.8.04	21	25	" "
35. Vince	25.8.04	14	51	" "
36. Hurrell	26.8.04	30	26	" "
37. Curry	27.8.04	11	21	" "
38. Hurrell	28.8.04	15	28	" "
39. Griffin	28.8.04	16	33	" "
40. Christie	29.8.04	14	19	" "
41. Martin	8.9.04	13	20	" "
42. Christie	8.9.04	15	29	" "
43. Fisher	8.9.04	15	46	" "
44. Campbell	9.9.04	22	27	" "
45. Christie	9.9.04	14	30	" "

Examination of Stools of Mediterranean Fever Cases—*contd.*

Name.	Dates.	Number of plates.	Day of disease.	Result.
46. Ingram.....	9.9.04	15	—	<i>M. melitensis</i> not isolated.
47. Groom.....	10.9.04	20	25	" "
48. Fisher.....	10.9.04	20	48	" "
49. Christie.....	10.9.04	18	31	" "
50. Groom.....	11.9.04	12	26	" "
51. Christie.....	11.9.04	11	32	" "
52. Fisher.....	11.9.04	15	49	" "
53. Groom.....	12.9.04	12	27	" "
54. Gane.....	12.9.04	12	23	" "
55. Christie.....	13.9.04	10	34	" "
56. Silcocks.....	13.9.04	10	36	" "
57. Jones.....	13.9.04	11	13	" "
58. Fisher.....	14.9.04	10	52	" "
59. Christie.....	14.9.04	10	35	" "
60. Silcocks.....	14.9.04	12	37	" "
61. ".....	15.9.04	10	38	" "
62. ".....	16.9.04	10	39	" "
63. Silburn.....	16.9.04	10	12	" "
64. Silcocks.....	17.9.04	20	40	" "
65. Hurrell.....	19.9.04	14	50	" "
66. Silcocks.....	19.9.04	14	42	" "
67. Fisher.....	19.9.04	20	57	" "
68. Barry.....	20.9.04	14	104	" "
69. Smith.....	20.9.04	14	25	" "
70. Silburn.....	20.9.04	14	16	" "
71. Jones.....	21.9.04	14	21	" "
72. Martin.....	21.9.04	12	33	" "
73. Iggo.....	21.9.04	12	11	" "
74. Rowlands.....	22.9.04	12	59	" "
75. Smith.....	22.9.04	12	27	" "
76. Rowlands.....	23.9.04	12	60	" "
77. Smith.....	23.9.04	12	28	" "
78. Silcocks.....	23.9.04	12	46	" "
79. Fisher.....	24.9.04	12	62	" "
80. Smith.....	24.9.04	22	29	" "
81. Rantiome.....	24.9.04	14	24	" "
82. Kinsella.....	25.9.04	16	30	" "
83. Anthony.....	25.9.04	14	18	" "
84. Smith.....	25.9.04	12	30	" "
85. Anthony.....	26.9.04	16	19	" "
86. Smith.....	26.9.04	16	31	" "

3. Examination of Sweat.

Critical perspirations, which are very characteristic of Mediterranean fever, have been examined at various periods of the disease, but the *M. melitensis* has not yet been isolated. The following examinations have been made:—

Experiment I.—On June 22, 1904, P . . . was noticed to be sweating profusely. The sweat was soaked up by means of sterile swabs which were then planted out in broth and rubbed over nutrose-agar plates

The tubes and plates were incubated at 37° C. On June 25, 1904, all the broth tubes showed a growth which was plated on nutrose-agar. The primary and secondary agar plates were carefully examined from time to time, but no signs of the *M. melitensis* could be discovered.

Experiment II.—At 8.30 p.m. on June 22, 1904, P . . . was again sweating profusely; swabs were treated as above, but the *M. melitensis* did not appear in the plates.

Experiment III.—At midnight on June 22, 1904, profuse sweats occurred in the same case, and the procedure detailed under Experiment I was followed. The *M. melitensis* was not isolated.

Experiment IV.—In the broth tubes, prepared as above, many contaminations were observed, which often rapidly overgrew the plates and so possibly prevented the *M. melitensis* from developing. In order to get rid of these extraneous organisms as far as possible the skin of P . . . was carefully washed with carbolic acid and ether, and a sterile pad covered by a sterile watch glass was bandaged on the right arm. On June 27, 1904, a critical sweat occurred, the pad was removed and planted out in broth; a growth occurred on June 29, 1904, which was found to consist of large Gram-staining cocci; no signs of the *M. melitensis* were discovered.

Experiment V.—On June 28, 1904, the procedure detailed under Experiment IV was followed in the case of H . . . large Gram-staining cocci again appeared.

Experiment VI.—On June 27, 1904, the same procedure was followed in the case of K . . . large and small Gram-staining cocci were isolated, but the *M. melitensis* did not appear.

Experiment VII.—On June 29, 1904, saturated pads obtained from P . . . were examined; the broth tubes remained absolutely sterile, although the incubation was continued for 10 days.

Experiment VIII.—On June 29, 1904, pads from Wildbore were planted out in broth. No growth resulted.

Experiment IX.—On June 29, 1904, pads from Wilson were planted out in broth. A growth occurred which, when plated, was found to give rise to large colonies, consisting of large cocci staining with Gram.

Experiment X.—On June 30, 1904, pads from Kelly were planted out in broth. No growth resulted.

It might be thought that the failure to obtain a growth recorded under Experiments VII, VIII, and X was possibly due to the presence in the swabs of carbolic acid, which, when transferred to the broth tubes, might inhibit the growth of the *M. melitensis*. In order to ascertain whether this was the case, sterile broth tubes, obtained in the manner detailed, were inoculated with *M. melitensis*. A typical growth resulted, showing that the failure to obtain a growth was not due to the presence of the disinfectant.

Experiment XI. Monkey No. 74.

To determine if the Injection of Sweat, from Malta Fever Patients, into a Monkey will give rise to the specific Fever.

The monkey arrived on August 29, 1904, and was taken at once to the roof of the Station Hospital, Valletta.

September 12, 1904. Skin scrapings were taken from the arms and axillæ of Private Lawrence, and ground up with normal salt solution. The resulting emulsion was injected subcutaneously into Monkey No. 74.

September 17, 1904. The blood was examined; the serum in a low dilution appeared to have a tendency to agglutinate the *M. melitensis*.

September 23, 1904. The blood was again examined, but the serum, diluted 1—10, did not show any signs of agglutinating the *M. melitensis*, even after waiting 1 hour.

September 25, 1904. Skin scrapings made into an emulsion with salt solution, were again injected.

September 27, 1904. Skin scrapings, treated as before, were injected.

September 28, 1904. The blood was examined, but the serum gave no reaction with the *M. melitensis*.

October 24, 1904. Staff-Surgeon Shaw continued the experiment up to this date. An agglutinative reaction was obtained with the serum, diluted 1—40, twenty-two days after the first injection.

The final result will be found in Dr. Shaw's experiments.

Experiment XII.

To determine if the Injection of Bacteria Free Sweat, derived from Malta Fever Patients, causes the Development of Agglutinins in the Blood of a Monkey.

Monkey No. 61A arrived in the laboratory on September 9, 1904. On September 15, 1904, and September 21, 1904, the serum was added in a low dilution to an emulsion of the *M. melitensis*; no trace of agglutination was observed.

September 22, 1904. Skin scrapings were taken from the arms and axillæ of Privates Kinsella and Silburn, who were suffering from Mediterranean Fever, and ground up with normal salt solution so as to form a fine emulsion. A sterile Berkefeld candle having been inserted into a sterile test-tube, the emulsion was filtered so as to remove all bacteria. The filtrate was then injected subcutaneously into Monkey No. 61A.

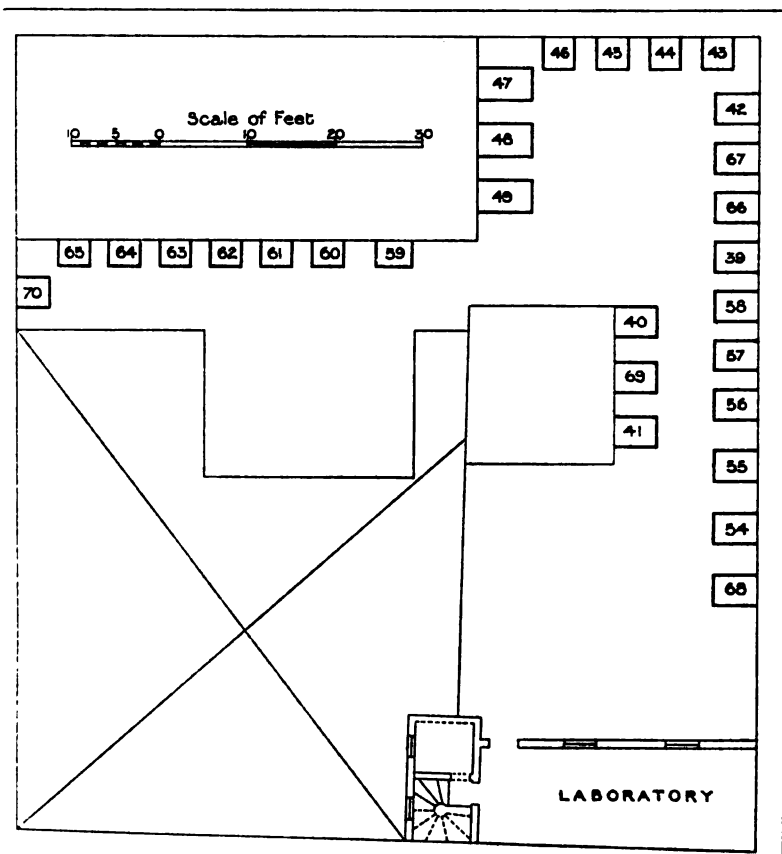
September 24, 1904. Sweat obtained from Privates Smith,

Silcocks, and Kinsella was similarly filtered, and the filtrate injected subcutaneously.

September 26, 1904. The blood was examined, and the serum found to have no action on the *M. melitensis*.

October 24, 1904. Dr. Shaw continued the experiment up to this date. The blood serum never caused the slightest agglutination of the *M. melitensis*.

Result.—The bacteria free filtrate obtained from the sweat of Malta fever patients does not appear to give rise to agglutinins in the blood of a monkey.



Plan of the Roof where Monkeys were kept, showing Position of the Animals which became naturally infected.

4. Examination of Expired Air of Malta Fever Patients.

In order to ascertain the presence of the *M. melitensis* in the expired air of Malta fever patients, a test-tube was fitted with an indiarubber

bung through which passed two glass tubes: one, attached to a mouth-piece, reached to the bottom of the test-tube and the other the exit tube, just passed through the bung. The test-tube was half-filled with nutrient broth and the whole apparatus then sterilised in the autoclave.

The patient under examination was directed to force expired air through the broth at frequent intervals throughout the day. The indiarubber bung, with glass tubes, was then removed, and the test-tube, being plugged with sterile cotton wool, was incubated at 37° C. After four days' incubation the broth was plated on nutrose-glucose-litmus-agar plates, and likely colonies fished and tested in the usual manner.

Case 1.—Private Markham breathed through one of these tubes on the 12.9.04; the tube was then incubated at 37° C. Four days later there was no sign of growth, but on the 19.9.04 a slight opalescence was noted. The broth was then plated on nutrose-glucose-litmus-agar. The plates were incubated for seven days, but no colonies of the *M. melitensis* appeared.

Case 2.—Private Lawrence breathed through a tube on the 12.9.04. On the 16.9.04 a marked growth appeared. A portion of the broth was plated as above, and the remainder of the growth injected into Monkey No. 73. After seven days' incubation no signs of *M. melitensis* could be discovered in the plates.

Case 3.—Private Markham again breathed through a tube on the 14.9.04. The tube was treated as before, and a slight growth was noticed on the 21.9.04. The growth was then plated, but no colonies of the *M. melitensis* appeared.

Case 4.—Private Lawrence breathed through a tube on the 14.9.04. On the 21.9.04 a slight growth appeared, which was then plated as before. No colonies of the *M. melitensis* were seen in the plates.

Case 5.—Private Kinsella breathed through a tube on the 17.9.04. On the 26.9.04 a slight growth appeared, but no colonies of *M. melitensis* were discovered in the plates made with the opalescent broth.

Case 6.—Private Silburn breathed through a tube on the 17.9.04. After twenty-four hours' incubation, the broth, being distinctly turbid, was plated in the usual manner, and incubation of the tube continued. Four days later a portion of the growth in the test-tube was plated out and the remainder of the growth injected into Monkey No. 73. No signs of the *M. melitensis* were discovered in the plates after prolonged incubation at 37° C.

Case 7.—Private Kinsella again breathed through a tube on the 20.9.04. No growth appeared in the broth, though incubation was continued for fourteen days.

Case 8.—Private Silburn breathed through a tube on the 20.9.04. A marked growth, having a putrefactive odour, appeared on the 24.9.04. This was then plated out as usual, but no colonies of the *M. melitensis* were discovered.

Case 9.—Private Silburn again breathed through a tube on the 23.9.04. The growth which appeared after incubation was treated in the usual manner, but no colonies of *M. melitensis* were isolated.

Case 10.—Private Tripp breathed through a tube on the 23.9.04. The tube was plated as before, but the *M. melitensis* was not isolated.

Case 11.—Private Anthony breathed through a tube on the 23.9.04. After the usual incubation the resulting growth was plated out, but with a negative result.

Case 12.—Private Rivers breathed through a tube on the 23.9.04. After the usual treatment, the *M. melitensis* was not isolated.

Monkey No. 73.

This monkey was reserved for the injection of broth infected by the expired air of Malta fever patients.

The monkey arrived at the laboratory on the 8.9.04. On the 15.9.04 a portion of its blood was removed and the serum, in a low dilution, added to an emulsion of the *M. melitensis*. No traces of agglutination were observed. On the 16.9.04 10 c.c. of broth infected by the breath of Private Lawrence were injected subcutaneously. On the 21.9.04 10 c.c. of broth infected by the breath of Private Silburn were injected. The action of the blood serum on the *M. melitensis* was also tested on this day, but no signs of agglutination were observed. On the 28.9.04 the blood serum was again examined, but no reaction with the *M. melitensis* was observed, though the dilution of the serum was only 1—10.

5. Examination of Sea-water in the Grand Harbour, Malta.

Having in view the result obtained when studying the viability of the *M. melitensis* in sea-water, and the fact that sea-water is extensively used for washing the decks of the battleships stationed in the Grand Harbour, it appeared desirable to ascertain whether the *M. melitensis* could be discovered in sea-water taken from this locality.

Studies of sea-water, when unsterilised and grossly infected with the *M. melitensis*, soon showed that the specific microbe could not be isolated, by ordinary bacteriological methods, a few days after the infection, owing to the saprophytic organisms overgrowing the colonies of the *M. melitensis*. Accordingly, it was decided to filter the sea-water through a sterile Berkefeld candle, and after washing the deposit with tap-water, to suspend it in 10 c.c. of tap-water, and inject the whole subcutaneously into a monkey.

On the 9.9.04 600 c.c. of sea-water, taken from the Grand Harbour opposite Fort St. Angelo, were pumped through a Berkefeld candle, and the deposit, having been well washed, was diffused in 10 c.c. of tap-water and injected subcutaneously into Monkey No. 71.

On the 10.9.04, the deposit from 600 c.c. of sea-water, taken from the same place, was injected.

On the 13.9.04, the deposit from 600 c.c. of sea-water, taken as before, was injected.

On the 15.9.04 the same procedure was followed.

On the 17.9.04 the same procedure was followed.

On the 18.9.04 the serum of Monkey No. 71 was added to an emulsion of the *M. melitensis*. No traces of agglutination were observed.

On the 19.9.04 600 c.c. of sea-water, taken off Fort St. Angelo, were again filtered, washed, and injected.

On the 21.9.04 the same procedure was followed.

On the 23.9.04 the same procedure was followed.

On the 25.9.04 1800 c.c. of sea-water were treated as before and the deposit injected. The serum of the monkey was added to an emulsion of *M. melitensis*, but no reaction was obtained.

On the 27.9.04 1800 c.c. of sea-water were filtered, and the washed deposit injected.

On the 29.9.04 600 c.c. of sea-water were treated as before. There is a small abscess at the site of the inoculation of the 27th.

Dr. Shaw continued this experiment up to October 22; the monkey received the bacteria contained in 30 litres of sea-water, but the blood serum never caused the slightest agglutination of the *M. melitensis*.

Result.—The *M. melitensis* could not be detected in the sea-water of the Grand Harbour.

4.

EXPERIMENTS ON THE MODE OF CONVEYANCE OF THE *MICROCOCCUS MELITENSIS* TO HEALTHY ANIMALS.

By Major W. H. HORROCKS, R.A.M.C., Member Mediterranean
Fever Commission.

(Received September 17, 1904.)

Experiment I.—Monkey No. 41.

*To Determine if the Inhalation of Dust, Infected with M. melitensis, will
give Rise to Mediterranean Fever in Healthy Monkeys.*

July 10, 1904. Monkey placed in cage and infected dust blown round him. Dust in bottle A used for this experiment, infected July 2, 1904.

July 11, 1904. Monkey kept in the cage and dust again blown round him. It was noticed, however, that owing to the moisture condensed on the walls, the dust soon settled, and it was impossible to keep it passing backwards and forwards through the cage. After an hour's interval, the cage was opened and the monkey allowed to come out into the room. Cage was then disinfected and dried.

July 12, 1904. Same procedure as July 10, 1904.

" 13, " " "

" 14, " " "

" 15, " " "

" 16, " " "

" 18, " " "

" 19, " " "

" 20, " " "

" 21, " " "

" 22, " Tested blood. No reaction.

" 23, " Placed in cage; dust blown as before.

" 25, " Placed in cage. The dust (bottle A) all expended.

Planted out one loop in broth to try and determine presence of *M. melitensis*. July 26, 1904, growth planted on agar; no signs of *M. melitensis*.

July 25, 1904. Prepared more dust to-day; dust (Petri dish half full) sterilised, and then inoculated with four agar slopes, third generation from spleen of man, dried over sulphuric acid *in vacuo*.

July 29, 1904. Monkey placed in cage and dust blown as before; dust dried over sulphuric acid employed.

July 31, 1904. Monkey placed in cage and dust blown as before; dust dried over sulphuric acid employed.

Note.—The dust appears to fall very rapidly; only seen on the nostrils. Mouth, as a rule, kept tight shut.

August 1, 1904. The same procedure as on July 29, 1904.

August 3, 1904. The same procedure as on July 29, 1904. Planted out soil in broth to see if *M. melitensis* still present; growth August 6, 1904, planted on agar. *M. melitensis* recovered.

August 4, 1904. The same procedure as on July 29, 1904; dust all expended.

August 5, 1904. Fresh dust prepared. Four tubes, second generation, spleen of Howe, incubated 3 days at 37° C., dried 24 hours over sulphuric acid and CaCl₂ *in vacuo*. Dust blown in cage. Dust planted out in broth on August 4, 1904, to ascertain presence of *M. melitensis*. August 8, 1904, planted on glucose agar; no *M. melitensis*, isolated; broth probably contaminated. This batch of broth found to be contaminated with *B. mesentericus*.

August 6, 1904. Dust blown in cage as on August 5, 1904. Dust planted out in broth August 9, 1904. Growth planted on agar August 10, 1904; broth contaminated, cause probably as on August 4, 1904.

August 8, 1904. Dust blown as before.

August 9, 1904. Planted out dust in broth (proved by incubation).
On August 13, 1904, growth planted on glucose-litmus-agar, *M. melitensis*
present.

August 10, 1904. Dust blown as before.

August 16, 1904. Examined blood; serum gave no reaction with *M. melitensis* in a dilution of 1 in 10.

August 26, 1904. Examined blood; serum reacted at once with *M. melitensis* in a dilution of 1 in 20; no reaction 1 in 50.

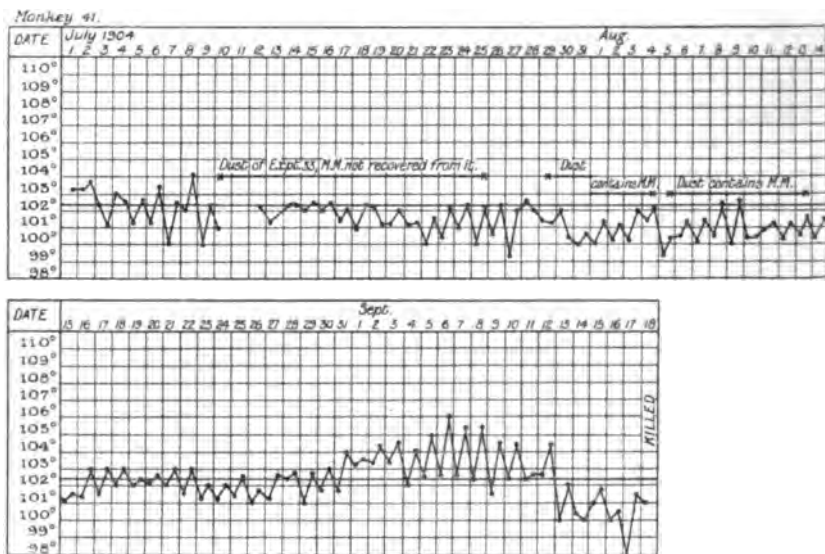
September 6, 1904. Examined blood; serum reacted at once, visible to naked eye, dilution 1—100; no reaction 1—500.

September 15, 1904. Examined blood; serum reacted at once, visible to naked eye, dilution 1—50; reaction incomplete in a dilution of 1—100.

September 19, 1904. Killed the monkey with chloroform. *Post-mortem* examination: Spleen enlarged, soft, and friable. Liver and kidneys congested. Made cultures from spleen, liver, and kidneys, urine, and heart's blood.

September 23, 1904. *M. melitensis* isolated from spleen of this monkey. Cultures made from liver, kidneys, and heart's blood are sterile.

The following chart represents the course of the *rectal* temperature :



Monkey No. 41.

Note.—The wave of fever did not commence until August 31, though a slight serum reaction was obtained on August 26. The first date on which the dust was known to contain the *M. melitensis* was July 29, consequently the incubation period might have varied from 17 to about 30 days.

Result.—This experiment seems to show that the inhalation or ingestion of infected dust will give rise to the disease.

Experiment II.—Monkey No. 47.

To determine if the Injection of Dust, infected with M. melitensis, into the Nostrils and Throat will give rise to Mediterranean Fever in Healthy Monkeys.

July 9, 1904. Injected dry dust containing *Micrococcus melitensis*, 7 days old, into both nostrils of above monkey. (Bottle A of July 2, 1904, used—Experiment 33.)

July 10, 1904. Injection repeated.

" 11, " "

" 12, " "

July 13, 1904. Injection repeated.

„ 14, „

„ 21, „ Examined blood; no reaction with *M. melitensis*.

„ 28, „

„ 29, „ Injected infected dust, dried 2 days over

acid *in vacuo*, into back of throat; lips covered with a cloth, and tube passed through a wooden gag.

July 30, 1904. Injection repeated as on July 29, 1904.

August 1, " " "

" 2, "

” 3, ” ” ”

” 4, ” ” ”

„ 5, „ Injection repeated, fresh dust prepared from four agar slopes, spleen Howe, second generation, incubated 3 days at 37° C., then dried for 24 hours over sulphuric acid and calcium chloride *in vacuo*.

August 6, 1904. Injection repeated as on August 5, 1904.

" 8, " Injection repeated. The greatest care is being taken to prevent abrasions of the mucous membrane ; a wooden gag is inserted between the teeth as before.

August 9, 1904. Examined blood; serum reacts completely to naked eye, dilution 1—40; slight reaction 1—80. No abrasions to be seen in the mouth; on the skin of the lower lip there is a very small abrasion, caused by the gag, but it is unlikely that this was the source of infection, as the lips have been covered as much as possible when the dust was blown.

August 16, 1904. Complete reaction at once 1—100, naked eye ; slight reaction 1—300.

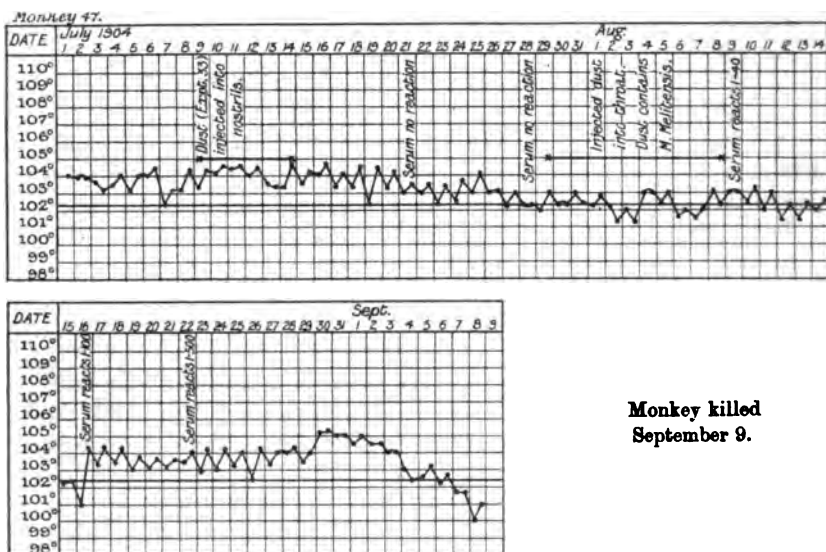
August 22, 1904. Examined blood, complete reaction at once 1—200; complete reaction, visible to naked eye in 10 minutes, dilution 1—500; 1—1000 dilution, *nil*.

September 9, 1904. This monkey has been very ill for some days, and has lost flesh rapidly. Being obviously in a dying state, he was killed with chloroform this morning. *Post-mortem* examination : Spleen enlarged, soft and friable. Kidneys markedly congested. Liver congested. Pericardium contained some fluid. Other viscera healthy.

Made cultures from the spleen, kidneys and liver.

The *M. melitensis* was not recovered, as all the cultures proved to be contaminated. The monkey was dying, and a batch of broth, which had not been tested by incubation, had to be used; unfortunately, all the broth tubes were found, on incubation, to be contaminated by *B. mesentericus*.

The following chart represents the course of the temperature:—



Monkey No. 47.

Result.—From this and the last experiment it is evident that the inhalation or swallowing of infected dust will give rise to Mediterranean Fever in monkeys.

Experiment III.—Monkey No. 39.

To determine if the Ingestion of Infected Food will give rise to Mediterranean Fever in Healthy Monkeys.

This monkey was kept under observation from July 1—10, 1904. It appeared perfectly healthy, and no cuts or abrasions were visible either on the body or in the mouth.

July 10, 1904. The growth from one agar slope, second generation, from spleen of man, and grown for 7 days at 37° C., was mixed with boiled potato, and eaten by the monkey.

July 11, 1904. The growth from one agar slope, as above, but grown for 8 days at 37° C., was mixed with boiled potato and two plums, and eaten by the monkey.

July 12, 1904. The same procedure followed, but the agar slope was 9 days old.

July 13, 1904. As on the 12th; growth 10 days old.

July 14, 1904. The same procedure followed, but a 9 days' old culture from heart's blood of a rabbit was employed.

July 15, 1904. Ten days' old culture, third generation, spleen of man used.

July 16, 1904. The same as on the 15th.

" 18, " " "

" 19, " Feeding continued as on July 15, 1904.

" 20, " Feeding continued as on July 15, 1904. Examined blood, serum diluted 1—10, gave no reaction with the laboratory strain of *M. melitensis*.

July 21, 1904. Feeding continued. One agar slope, first generation, spleen H—, incubated for 7 days at 37° C., used.

July 22, 1904. Feeding continued. One agar slope, first generation, kidney H—, incubated for 8 days at 37° C., used.

July 23, 1904. The feeding was continued, but I omitted the plums from the mixture, as I found they gave rise to a strongly acid reaction which might inhibit or destroy the *M. melitensis*. One agar slope, first generation, kidney of H—, incubated for 9 days at 37° C., was employed.

July 25, 1904. Half an agar tube of third generation, spleen of H—, was given. The blood was examined for agglutination, but the serum, diluted 1—10, gave no reaction with the *M. melitensis*.

July 26, 1904. Half an agar tube of third generation, spleen of H—, incubated for 4 days at 37° C., was employed.

July 27, 1904. One agar slope, third generation, spleen of H—, incubated for 14 days at 37° C., mixed with potato.

July 28, 1904. One agar slope, fourth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

July 29, 1904. One agar slope, fourth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

July 30, 1904. One agar slope, fifth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

August 1, 1904. One agar slope, fifth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

August 2, 1904. One agar slope, fifth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

August 3, 1904. One agar slope, second generation, spleen of H—, incubated for 3 days at 37° C., mixed with potato. Only a small portion was consumed.

August 4, 1904. One agar slope, fourth generation, spleen of H—, incubated for 5 days at 37° C. Only a small portion was eaten.

August 5, 1904. One agar slope, second generation, from urine of Sergeant P—, and incubated 10 days at 37° C., mixed with potato.

August 6, 1904. One agar slope, third generation, spleen of H—, incubated for 72 hours at 37° C., mixed with potato.

August 8, 1904. One agar slope, third generation, spleen of H—, incubated for 6 days at 37° C., mixed with potato.

August 9, 1904. One agar slope, third generation, spleen of H—, incubated for 6 days at 37° C., mixed with potato.

August 10, 1904. One agar slope, third generation, spleen of H—, incubated for 15 days at 37° C., mixed with potato. Examined blood; serum reacts at once, visible to the naked eye, in a dilution of 1—80; under the microscope reaction is seen at once with a dilution of 1—160.

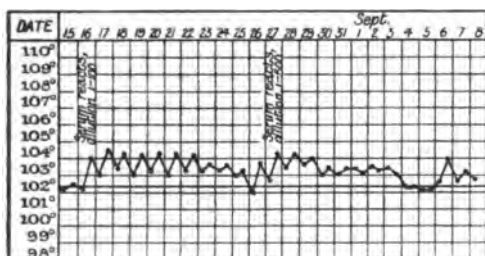
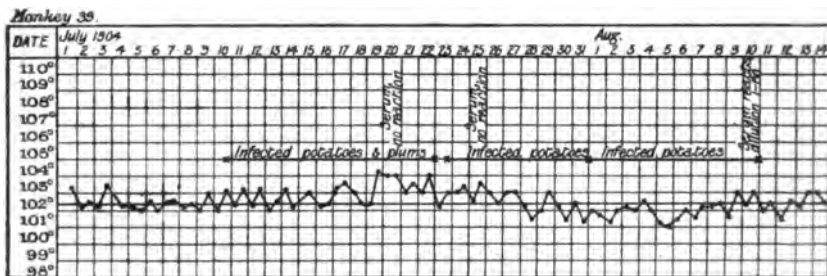
August 16, 1904. Examined blood; serum reacts at once, visible to the naked eye, dilution 1—100. Dilution 1—300 shows a reaction under $\frac{1}{2}$ th.

August 27, 1904. Examined blood; serum reacts at once, visible to the naked eye, dilution 1—100. After 5 minutes, dilution 1—500, is visible to the naked eye.

September 8, 1904. Killed the monkey with chloroform. Body well nourished. *Post-mortem*. Spleen enlarged, soft and friable. Kidneys congested. Liver congested. Other viscera normal.

September 14, 1904. Recovered *M. melitensis* from the spleen.

The following chart represents the temperature curve :—



Monkey killed
September 8.

Monkey No. 39.

Result.—The absorption of the *M. melitensis* was extremely slow, but the monkey eventually suffered from an acute infection.

Experiment IV.—Monkey No. 40.

To determine if the Ingestion of Infected Food will give rise to Mediterranean Fever in Healthy Monkeys.

July 10, 1904. Half of the potato prepared for Monkey No. 39 was given to this monkey. The dose of *M. melitensis* corresponded to one agar slope, as in the case of Monkey No. 39.

July 11, 1904. The same procedure was followed as in Experiment III, Monkey No. 39.

"	12,	"	"	"	"
"	13,	"	"	"	"
"	14,	"	"	"	"
"	15,	"	"	"	"
"	16,	"	"	"	"
"	18,	"	"	"	"
"	19,	"	"	"	"
"	20,	"	"	"	"
"	21,	"	"	"	"
"	22,	"	"	"	"
"	23,	"	"	"	"
"	25,	"	"	"	"

The same procedure was followed as in Experiment III, Monkey No. 39. Examined blood; serum gave no reaction with *M. melitensis*.

" 26, " The same procedure was followed as in Experiment III, Monkey No. 39.

"	27,	"	"	"	"
"	28,	"	"	"	"
"	29,	"	"	"	"
"	30,	"	"	"	"

August 1, " " " " "

" 2, " " " " "

" 3, " " " " "

" 4, " " " " "

" 5, " " " " "

" 6, " " " " "

" 8, " " " " "

" 9, " " " " "

" 10, " " " " "

" 11, " Examined blood. Complete instantaneous agglutination, visible to the naked eye, dilution 1—30. After standing 5 minutes, dilution 1—100; was also visible to the naked eye.

August 20, 1904. Examined blood. Serum gave a reaction with *M. melitensis* when diluted 1—10, but no result was obtained with higher dilutions.

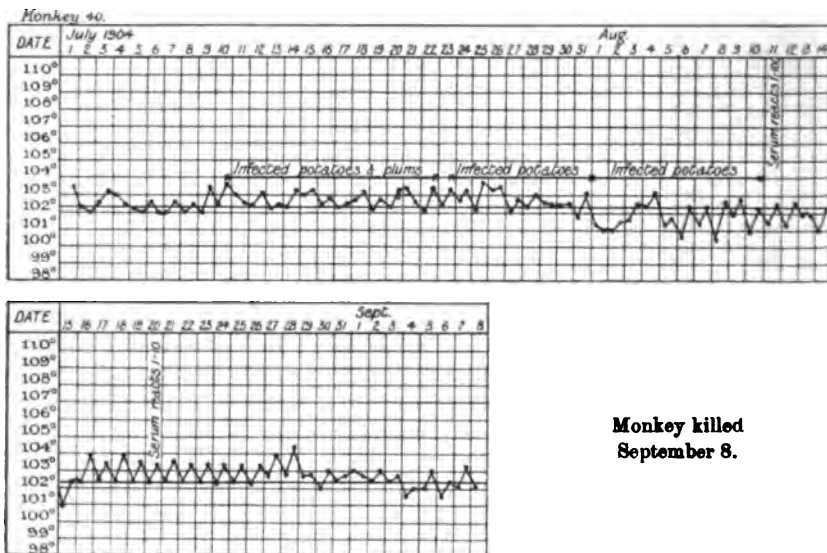
September 8, 1904. Monkey killed by chloroform. *Post-mortem* :

Spleen enlarged, but not so markedly as No. 39; kidneys congested; other viscera apparently healthy. Made cultures from the spleen, liver, and kidneys.

September 16, 1904. *M. melitensis* not recovered from the cultures made at the *post-mortem* examination. All the cultures proved to be sterile.

Note.—It seems probable that, in the case of this monkey, the bacterial infection was never marked, and that the few micrococci absorbed might easily have been destroyed.

The following chart represents the temperature curve:—



Monkey No. 40.

Experiment V.—Monkey No. 66.

To determine if the Ingestion of Infected Food will give rise to Mediterranean Fever in Healthy Monkeys.

August 13, 1904. This monkey is in a box next to Monkey No. 39, and I noticed about a week ago that he ate some of the infected potato provided for No. 39. Examined blood, serum reacts instantaneously, visible to naked eye, dilution 1—100. Visible under $\frac{1}{12}$ th after 10 minutes in a dilution of 1—500.

August 18, 1904. Believing this monkey to be healthy, Dr. Zammit, at 6.30 P.M. last evening, injected a small quantity of blood from a Mediterranean Fever patient. In order not to vitiate both experiments the monkey was killed at 11 this morning.

Post-mortem examination :—

Abdomen : Spleen enlarged and congested. Kidney enlarged and congested. Liver congested. Intestines appeared normal.

Thorax : Lungs healthy. Heart appeared dilated.

Cultures made as follows :—

Spleen : (a) Planted out in broth and (aa) rubbed over an agar slope.

(b) Kidney planted out in broth and (bb) rubbed over an agar slope.

(c) Liver planted out in broth.

(d) Heart's blood, planted out in two broth tubes.

(e) Urine, planted out in broth.

August 21, 1904. Typical colonies have appeared on the agar slope, made from the spleen ; fished one—it agglutinated at once with serum from Monkey No. 45.

August 22, 1904. Planted out colony from spleen on an agar slope.

„ growth in broth, from heart's blood
(two tubes), on an agar slope.

„ growth in broth, from kidney, on an
agar slope.

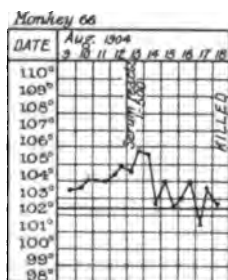
„ growth in broth, from liver, on an
agar slope.

August 24, 1904. Typical growth obtained from colony of spleen, planted out in litmus milk and glucose. Litmus milk rendered alkaline, glucose not fermented.

August 26, 1904. Typical growth, agglutinating at once with dilute serum, obtained from heart's blood.

August 28, 1904. Typical growth, agglutinating at once with monkey serum, obtained from kidney.

The following chart represents the temperature curve :—



Monkey No. 66.

Note.—This experiment is probably an instance of direct absorption of *M. melitensis* through a crack or abrasion of the mucous membrane of the mouth. The period of incubation and the wave of fever correspond exactly with those of Monkey No. 72, which was infected with

M. melitensis through a crack in the mucous membrane over the incisor teeth.

Experiment VI.—Monkey No. 72.

To Differentiate between Absorption from the Mouth and Throat and Absorption from the Stomach and Intestines.

Monkey No. 72 arrived in the laboratory on September 10, 1904. The blood was tested and gave no reaction with the *M. melitensis*. Feeding was then commenced, infected milk being passed directly into the stomach by means of an indiarubber tube. The growth on one agar slope, second generation, spleen of H—, incubated for 6 days at 37° C., was employed.

September 13, 1904. The feeding was continued as before, the growth on one agar slope, incubated for 7 days, being given. A small quantity of the milk regurgitated into the mouth, but no abrasion could be seen on the mucous membrane.

September 14, 1904. The growth from one agar slope, incubated for 8 days, was given.

September 15, 1904. The feeding was continued as before.

September 16, 1904. The feeding was continued, the growth from one agar slope, incubated for 5 days, being given. A little milk again regurgitated into the mouth, and, on examination, a small crack was found in the mucous membrane opposite the upper incisor teeth. The mouth was at once washed out with lysol. The blood was examined but no reaction with the *M. melitensis* was obtained.

September 17, 1904. The feeding was continued, the growth from one agar slope, incubated for 5 days, being given.

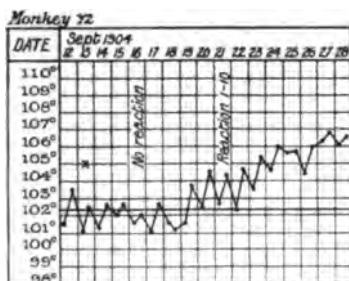
September 18, 1904. The growth from one agar slope, incubated for 6 days, was given.

September 21, 1904. The blood was examined and the serum in a dilution of 1—10, caused instantaneous clumping of the *M. melitensis*.

September 26, 1904. The serum, diluted 1—100, was found to agglutinate the *M. melitensis* instantaneously, the reaction being visible with the naked eye.

Note.—This monkey was directly infected either on September 13 or 16, the short incubation and sharp rise of temperature correspond to what is seen when the *M. melitensis* is directly absorbed into the peripheral circulation. Owing to the regurgitation of the infected milk into the mouth the experiment failed to differentiate between absorption from the mouth and from the alimentary canal; it, however, explains what probably occurred in the case of Monkey No. 66.

The prolonged incubation or rather slow absorption observed in the case of Monkeys Nos. 39, 40, and 41 forms a marked contrast to the rapid infection noticed in Monkeys Nos. 66 and 72, and approximates very closely to the results obtained when human beings are infected under natural conditions.



Monkey No. 72.

Experiment VII.—Monkey No. 45.

To note the Effect of the Subcutaneous Inoculation of M. melitensis in Healthy Monkeys, and to Obtain a Specific Serum.

July 9, 1904. Injected $\frac{1}{2}$ c.c. of emulsion from an agar tube, second generation, from spleen of man. The agar tube was incubated for 6 days at 37° C., and the whole of the growth was used for the emulsion.

July 15, 1904. Complete agglutination with *M. melitensis* serum, diluted 1—10, and up to 1—160. No reaction with a dilution of 1—300.

July 21, 1904. Monkey looks ill. Tested serum—complete reaction, naked eye at once, dilution 1—1000. Shaved hair on back, and Zammit applied two female *Stegomyia*, which fed voraciously.

July 22, 1904. Zammit's feeding experiments with mosquitoes continued.

July 23, 1904. Zammit's feeding experiments with mosquitoes continued.

July 26, 1904. Tested serum; complete agglutination to naked eye, within 1 minute, dilution 1—1000.

August 1, 1904. Tested serum; complete agglutination to naked eye, within 1 minute, dilution 1—1000.

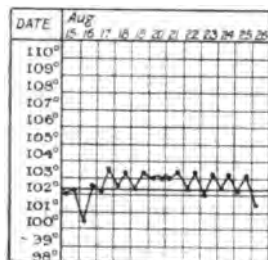
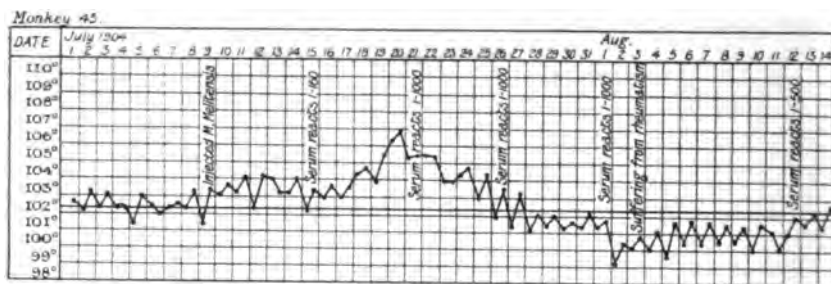
August 3, 1904. Monkey suffering from rheumatism (?); right arm and right wrist joint painful.

August 12, 1904. Examined blood; serum reacted at once, visible to naked eye, dilution 1—100; reaction after 5 minutes, dilution 1—500; dilution 1—1000, no reaction 5 minutes; feeble reaction, under microscope, after $\frac{1}{2}$ hour.

September 9, 1904. Killed the monkey with chloroform. *Post-mortem*: Spleen much enlarged. Liver and kidneys congested. Other viscera healthy. Made cultures from the spleen, kidney and liver.

September 13, 1904. Recovered *M. melitensis* from spleen.

The following chart represents the temperature curve:—



Monkey No. 45.

Result.—The monkey suffered from a typical attack of Mediterranean fever.

Experiment VIII.—Monkey No. 48.

To Note the Effect of the Injection of Washings of Dust derived from Sergeants' Mess, Melleha Camp.

July 16, 1904. Dried soil (dust) from ventilation aperture, between w.c. and dining room of sergeants' mess at Melleha, received from Dr. Johnstone.

Soil macerated in sterile water, filtered, soil remaining washed, filtrate treated as follows:—10 c.c. injected into Monkey No. 48, subcutaneously between shoulders.

July 18, 1904. Ten cubic centimetres of further washings injected.

July 23, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

August 11, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

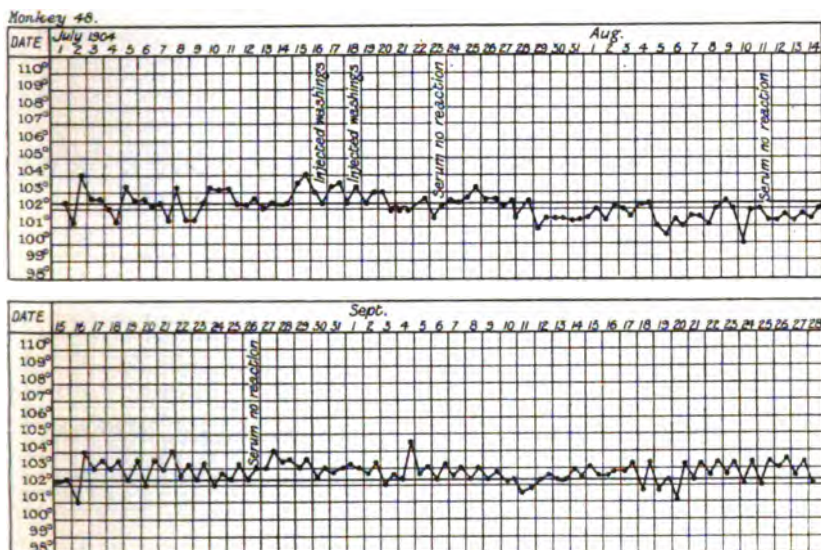
August 26, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

September 6, 1904. Examined blood ; no reaction with *M. melitensis*, dilution 1—10.

This experiment was performed at the request of Dr. Johnstone. The sergeants' mess at Melleha appeared to be the probable centre of infection of the sergeants of the Essex regiment. A disused w.c. was

found communicating by a ventilating aperture with the mess room. The dust was derived from this ventilating aperture.

The following chart represents the temperature curve :—



Monkey No. 48.

Result.—The *M. melitensis* was not present in the dust removed from the ventilating aperture.

Experiment IX.—Monkey No. 43.

To Note the Effect of the Injection of Washings of Supposed Infected Soil into a Healthy Monkey.

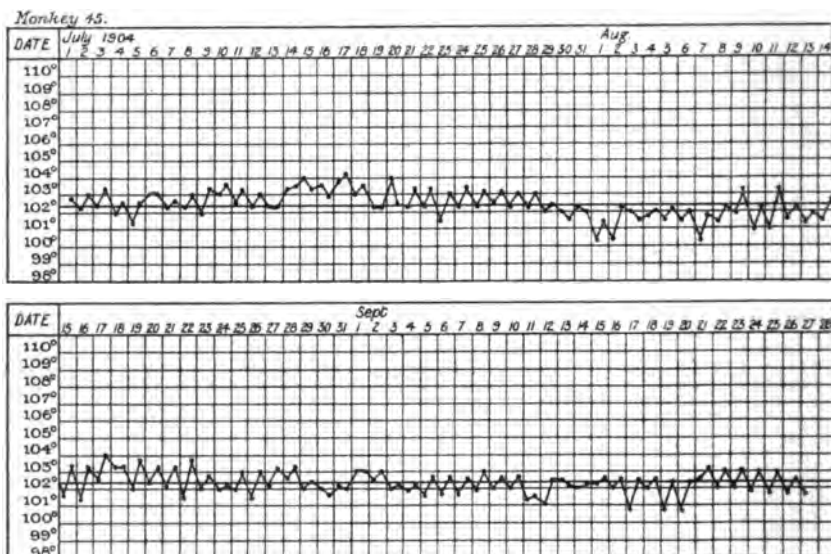
July 16, 1904. Dr. Johnstone forwarded 0·14 gramme of soil, obtained from the pan of the disused w.c. in the sergeants' mess, Melleha Camp. The soil was macerated in sterile water, filtered through paper, and the deposit again thoroughly washed. The total filtrate obtained was 20 c.c. Of this 10 c.c. was injected subcutaneously into a monkey.

July 18, 1904. The remainder of the washings injected.

July 23, 1904. Examined blood; serum, diluted 1—10, gave no reaction with *M. melitensis*.

August	1,	"	"	"	"
"	10,	"	"	"	"
"	17,	"	"	"	"
"	26,	"	"	"	"
September	6,	"	"	"	"

The following chart represents the temperature curve:—



Monkey No. 43.

Result.—The *M. melitensis* was not present in the soil removed from the w.c. in the sergeants' mess.

Experiment X. Monkey No. 46.

Injection of Washings from Wall of an Infected House.

July 7, 1904. The walls of the w.c., No. 26 Strada Nuova, Sliema, where two cases of Mediterranean Fever occurred, were rubbed with cotton wool moistened with saline solution; the water was expressed and filtered through paper. Filtrate, collected in a sterile tube, was treated as follows:—

Injected 10 c.c. of filtrate.

July 8, 1904. Injected 10 c.c. of filtrate.

July 9, 1904. " "

July 10, 1904. Injected the remaining portion (8 c.c.) of filtrate.

July 16, 1904. Examined blood, no reaction with *M. melitensis*, dilution 1—10.

July 18, 1904. Washings from kitchen, grown in broth for 11 days, injected to-day.

July 26, 1904. Tested serum, no reaction with *M. melitensis*, dilution 1—10.

August 10, 1904. Examined serum, no reaction with *M. melitensis*, dilution 1—10.

September 6, 1904. Examined blood; serum reacts at once with *M. melitensis*, dilution 1—10; reaction 1—500, after waiting 15 minutes.

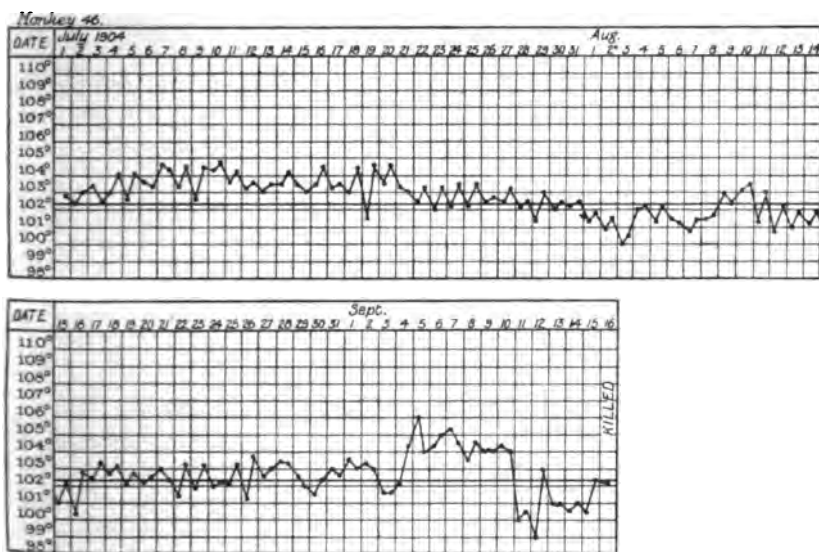
September 15, 1904. Examined blood; serum reacts at once in a dilution of 1—500; in a dilution of 1—1000 a reaction, visible to the naked eye, is seen in 5 minutes.

September 16, 1904. Killed the monkey with chloroform.

Post-mortem.—Spleen enlarged, but firm in consistence; kidneys and liver congested; pericardium contained a little fluid; other viscera healthy. Cultures made from spleen, liver, kidney, heart's blood, and urine.

September 23, 1904. *M. melitensis* isolated from spleen, kidney, and urine.

The following chart shows the temperature curve:—



Monkey No. 46.

Remarks.—This result is probably due to infection conveyed from neighbouring monkeys. Even if the *M. melitensis* had been present in the growth injected on July 18, it is highly improbable that the specific microbe when injected subcutaneously would have remained latent for a period of 50 days. Monkey No. 69 has also become infected since its arrival, without receiving the specific microbe either by the mouth or subcutaneously.

Monkey No. 46 on one side is next to Monkey No. 45, which received *M. melitensis* subcutaneously and developed a typical attack of fever.

On the other side of Monkey No. 46 is Monkey No. 47, infected by dust blown into the throat. Evidently this monkey has become infected, either by personal contact, by urine, or by means of *Stegomyia*.

Experiment XI.—Monkey No. 42.

To Determine if the Subcutaneous Injection of Infected Urine from a Case of Mediterranean Fever will give rise to the Disease in a Monkey.

July 13, 1904. Injected 10 c.c. of Howe's urine, enriched with broth, and incubated for 14 days at 37° C. (3 c.c. urine).

July 14, 1904. Injected 10 c.c. of Howe's urine (3 c.c. urine) treated as above, but incubated 15 days.

July 15, 1904. Injected 10 c.c. of mixed urine and broth (3 c.c. of urine), incubated 14 days.

July 18, 1904. Examined blood. Feeble reaction with one culture, blood diluted 1—10; tested with another culture, no reaction was obtained.

July 19, 1904. Injected 5 c.c. of broth culture, made at *post-mortem* of Howe by adding 1 c.c. of urine from bladder to broth, and then incubating at 37° C. for 12 days. Examined by hanging drop; fine cocci and chains, corresponding in morphology to *M. melitensis*, observed, the cocci decolorised by Gram.

July 20, 1904. Injected 10 c.c. of broth culture, made at *post-mortem* by adding contents of right ureter to a broth tube.

July 25, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

August 2, „ „ „ „

„ 11, „ „ „ „

„ 26, „ Examined blood; reacts 1—10 at once.

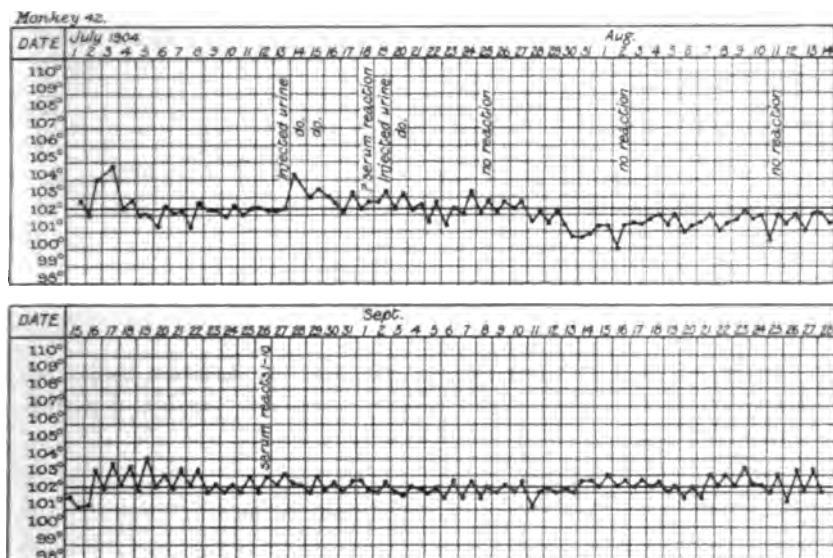
September 7, 1904. Examined blood; serum reacts in dilution of 1—20 at once: dilution 1—100, no reaction.

September 27, 1904. Killed monkey; made cultures from spleen, liver, kidney, heart's blood, and urine.

October 10, 1904. All the cultures have remained sterile.

Note.—The *M. melitensis* was recovered by plating another sample of the urine, removed from the bladder at the *post-mortem*.

The following chart shows the temperature curve; it will be noticed that there has never been a wave of fever, the slight serum reaction was probably caused by toxins contained in the urine:—



Monkey No. 42.

Remarks.—The *M. melitensis* was probably not present in the specimens of urine injected into this monkey. The slight blood reaction obtained might be caused by toxins in the urine.

Experiment XII.—Monkey No. 55.

To Determine whether Cultures of M. melitensis, Derived from Infected Urine, will give Rise to the Disease in a Monkey.

July 29, 1904. Growth from Pudney's urine, third generation, grown for 3 days on agar slope (glucose-litmus-nutrose-agar). The whole of the growth diffused in 2 c.c. of broth, and injected into this monkey.

August 4, 1904. Examined blood; complete instantaneous reaction, visible to naked eye, blood dilution 1—10; no reaction 1—50.

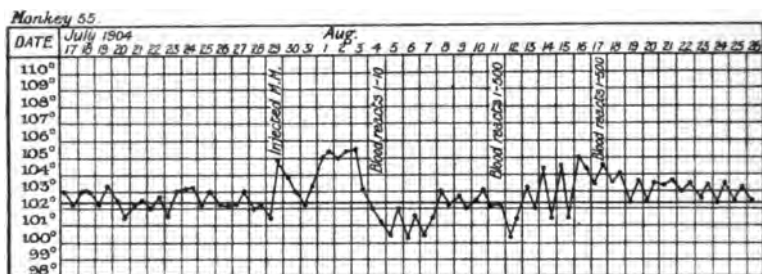
August 11, 1904. Examined blood; complete instantaneous reaction visible to naked eye, dilution 1—100; after 5 minutes, reaction visible in dilution 1—500.

August 17, 1904. Examined blood; reaction as on August 11, 1904.

September 8, 1904. Killed the monkey to-day. *Post-mortem*: Spleen enlarged and friable. Kidneys congested. Other viscera apparently healthy. Made cultures from the spleen, liver and kidneys.

September 12, 1904. *M. melitensis* recovered from the spleen.

The following chart shows the temperature curve:—



Monkey No. 55.

Result.—This experiment shows that the *M. melitensis* recovered from the urine of Mediterranean Fever patients is capable of giving rise to the disease in healthy monkeys.

Experiment XIII.—Monkey No. 69.

Is Mediterranean Fever Conveyed from Diseased to Healthy Monkeys by Contact?

This monkey arrived in the laboratory on August 7, 1904. It was placed in a cage, between Monkey No. 41, infected by dust, and Monkey No. 40, infected by feeding. Monkey No. 69 appeared perfectly healthy on arrival, and ate well; its temperature was taken from August 9, and after August 16 displayed an erratic course, probably accounted for by the intense heat of the terrace from early morning until evening.

On August 26, 1904, the blood was examined, but the serum, diluted 1—10, gave no signs of reaction with *M. melitensis*.

On September 7, 1904, the blood was again examined, and the serum, diluted 1—10, caused immediate clumping of the *M. melitensis*, visible to the naked eye.

Since September 9, 1904, the monkey has been obviously ill, losing flesh and sitting "moping" in his box all day.

On September 11, 1904, the serum, diluted 1—20, caused instantaneous clumping of the *M. melitensis*.

On September 13, 1904, the monkey died, much emaciated.

Post-mortem examination: All the viscera appeared healthy; cultures were made from the spleen, liver, kidneys, heart's blood and urine. *M. melitensis* isolated from the spleen and liver.

Remarks as to the Mode of Infection of this Monkey.—It seems possible that it might have occurred in three ways, i.e. (a) by direct personal contact; (b) by direct infection from walking in the infected urine of his neighbours; (c) by means of *Stegomyia*. When at full

length of his chain, Monkey No. 69 could touch either of his neighbours and walk on the ground infected by them.

If personal contact alone had been the cause of infection, Monkey No. 48 ought to have been infected by Monkey No. 47. Also the *M. melitensis* has not yet been isolated from the sweat or skin scrapings of patients suffering from Mediterranean fever.

If the infection had been carried by *Stegomyia*, there should have been a general infection amongst the monkeys on the terrace. There appears no reason why mosquitoes should have picked out Monkey No. 69 and Monkey No. 46, which also appears to have been infected by its neighbours. At this time there were six other healthy monkeys on the terrace exposed to the bites of mosquitoes, and one of them, No. 48, was in a cage next to an infected monkey. Yet none of these monkeys have shown the slightest trace of a blood reaction. Direct infection through infected urine seems to be the most probable explanation of the infection. Both Monkey No. 69 and Monkey No. 46 had infected monkeys next to them, and the chance of infection from urine was undoubted, as the *M. melitensis* was discovered in the urine of Monkey No. 46, proving that the specific microbe is excreted from monkeys in the same manner as from human beings. Although the cages and cemented surfaces beneath them were washed with lysol night and morning, still the ground was often noticed covered with decomposing urine.

Having in view the possibility of direct infection from urine excreted by monkeys suffering from Mediterranean fever, it is necessary to enquire whether any of the experiments previously recorded are invalidated by this circumstance. It will be advisable to discuss the experiments *seriatim*.

Experiment I, Monkey No. 41.—This monkey was kept in a small room on the left of the door leading from the laboratory to the roof. It was not placed in its box until infection had been acquired, and even after this it was still separated from Monkey No. 40 by a healthy monkey. It is evident that in relation to this experiment the question of infection by urine could not arise.

Experiment II, Monkey No. 47.—This monkey was placed between two healthy monkeys, viz., No. 46 and No. 48. Monkey No. 48 remained in good health throughout the summer and never showed the slightest sign of infection. Monkey No. 47 was infected on August 8, 1904, but Monkey No. 46 did not show a reaction until September 6, 1904. It is obvious that Monkey No. 47 could not have been infected by urine excreted by its neighbours.

Experiment III, Monkey No. 39.—The monkey was placed between Monkey No. 58 and Monkey No. 66. Monkey No. 58 only received injections of filtered toxines, and could not possibly excrete the specific micrococci in its urine. Monkey No. 66 was directly infected through

a crack in the mouth, and suffered from a marked bacterial infection ; its first rise of temperature occurred on August 10, 1904, and it is practically impossible that the *M. melitensis* could have been excreted in its urine before this date, and, taking into consideration the facts observed in man, it is unlikely that the urine would contain the *M. melitensis* before August 25, 1904. Consequently it seems impossible that the Monkey No. 39 could have received infection from the urine of its neighbours.

Experiment IV, Monkey No. 40.—This monkey was infected on August 11, 1904, and the monkeys nearest to it, viz., 69 and 41, were not infected until September 7, 1904, August 26, 1904, respectively. The question of infection by urine could not arise in this case.

Experiment V, Monkey No. 66.—This monkey was placed between Monkey No. 67 and Monkey No. 39. Monkey No. 67 never showed the slightest trace of infection, and was in good health all the summer. Monkey No. 39, as previously stated, was infected about the same date as Monkey No. 66. It does not seem possible that infection by urine could have played a part in this experiment.

Experiment VI, Monkey No. 72.—This monkey was directly infected through a crack in the mucous membrane of the mouth on September 13 or 16. It was kept apart from infected monkeys.

Experiment VII, Monkey No. 45.—This monkey was directly infected by subcutaneous injection of the *M. melitensis*.

Experiment VIII, Monkey No. 48 } These monkeys failed to become
Experiment IX, Monkey No. 43 } infected.

Experiment X, Monkey No. 46.—This monkey was infected on September 6, 1904, and it appears practically certain that the infection was caused by the specific micrococci present in the urine of neighbouring monkeys.

Experiment XI, Monkey No. 42.—This monkey probably only received toxins contained in the urine excreted by a case of Mediterranean fever.

Experiment XII, Monkey No. 55.—This monkey was directly infected by the subcutaneous injection of the *M. melitensis*.

Experiment XIII, Monkey No. 69.—This monkey became infected on September 7, 1904. The source of infection was probably the urine of its neighbours.

List of Monkeys, not infected, artificially infected, and naturally infected, with Dates of Arrival and Infection.

No.	Infection.	Arrival.	Remarks.
70.	Not infected.	8/8/04.	
65.	"	"	Dr. Zammit's mosquito experiments.
64.	"	"	" "
63.	Artificially infected.	"	" "
62.	Not infected.	16/7/04.	
61.	"	"	
60.	"	"	Died, diarrhoea, 26/8/04.
59.	"	"	Died 11/9/04.
49.	"	8/8/04.	
48.	"	1/7/04.	
47.	Artificially infected 9/8/04.	"	Died. Experiment II, page 48.
46.	Naturally infected 6/9/04.	"	
45.	Artificially infected 15/7/04.	"	Subcutaneous injection 9/7/04.
44.	Not infected.	"	Died from pneumonia, 6/7/04.
43.	"	"	
42.	(?) Infected (probably toxine)	"	Urine infection.
37.	Not infected.	8/8/04.	Mosquito experiment.
36.	Artificially infected P 9 or 10/8/04.	"	Food experiment. Serum 18/8/04.
39.	Artificially infected 10/8/04.	1/7/04.	Food experiment.
58.	Not infected.	16/7/04.	Toxine injected.
57.	"	"	Died from diarrhoea 15/8/04.
56.	"	"	5/8/04.
55.	Infected 4/8/04.	"	Culture from urine. Serum 4/8/04.
54.	Not infected.	"	Skin scraping.
68.	"	8/8/04.	"
40.	Artificially infected 11/8/04.	1/7/04.	Experiment IV, page 53.
69.	Naturally infected 7/9/04.	8/8/04.	
41.	Artificially infected 26/8/04.	8/7/04.	Died. Experiment I, page 46.

MOSQUITO EXPERIMENTS.

These experiments were undertaken in order to ascertain whether the *Stegomyia fasciata* is able to convey the *M. melitensis* from the peripheral blood of Malta Fever patients to healthy monkeys.

Experiment I.

In this experiment the mosquitoes were fed on Private Lawrence, 2nd Essex Regiment. This particular patient was selected, as Staff-Surgeon Shaw had found the maximum number of micrococci in his blood. The number of mosquitoes and the dates on which they were

fed on the patient and on Monkey No. 70, are shown in Table I. An endeavour was made to keep the mosquitoes alive as long as possible, as in view of the work done on Yellow Fever it seemed possible that several days might intervene between the absorption of the *M. melitensis* into the stomach of the mosquito and its transfer, possibly through the salivary glands, to the proboscis. In Dr. Zammit's successful experiment only 48 hours intervened between the absorption of the micrococci and their transfer to the patient. In Experiment I the intervals were 2, 4, 8, and 10 days, respectively. Monkey No. 70 had been under observation for several months and always appeared perfectly healthy. Its serum was examined at varying periods, but it never manifested the slightest power of agglutinating the *M. melitensis*.

Experiment II.

The same procedure was followed in this experiment, the patient, Private K—, R.A.M.C., having a typical wave of fever. The number of mosquitoes and the dates when they were fed on the patient and on Monkey No. 44, are given in Table II. The mosquitoes were kept alive for 13 days, and yet no trace of agglutination could be detected when the serum of the monkey, in a low dilution, was added to an emulsion of the *M. melitensis*.

Experiment III.

In this experiment mosquitoes were fed on different patients, specially selected owing to the presence of marked fever at the time of feeding. The details of the various feedings are given in Table III. All the agglutination tests were negative.

Experiment IV.

In this experiment mosquitoes were fed on monkeys recently inoculated with *M. melitensis* and, after an interval of 48 hours, transferred to Monkey No. 76 which arrived at the laboratory on 8.9.04. On 16.9.04 and 22.9.04 the serum of Monkey 76, diluted 1—10, was added to an emulsion of *M. melitensis*; no agglutination was observed on either occasion. On the 20.9.04 mosquitoes were fed on Monkey No. 60A, at that time at the summit of a wave of fever, and 48 hours later they were fed on Monkey No. 76. On the 25.9.04, mosquitoes were again fed on Monkey No. 60A, and on the 27.9.04 transferred to Monkey No. 76. On the 27.9.04 mosquitoes were fed on Monkey No. 72, infected by feeding and at the height of a wave of fever, and 48 hours later transferred to Monkey No. 76. The serum of Monkey No. 76 was examined on 27.9.04, but did not manifest the slightest power of agglutinating the *M. melitensis*.

(These experiments are still proceeding.)

Table II.—Monkey No. 44. Mosquito Experiments (continued up to the end of October).

Mosquitoes fed on patient.		Mosquitoes fed on monkey.																				
Date.	No. of mosquitoes.	No. of days after being fed on patient.																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1904.																						
Aug. 29..	1	..	1
" 31..	2	..	1
Sept. 14..	1	..	1
	1	..	1
	4	..	1
" 17..	1	..	1
	1	..	1
	2	..	1
Oct. 5..	1	..	1
" 5..	1	..	1
	1	..	1
	4	..	1
" 5..	1	..	1
	1	..	1
	2	..	1
" 24..	1	..	1
	1	..	1
	4	..	1
	1	..	1

This monkey was bitten 93 times by presumably infected mosquitoes, and in the case of two mosquitoes 21 days intervened between the first feeding on the patient and the last feeding on the monkey. Its serum was repeatedly examined, but never caused the slightest agglutination of the *M. melitensis*.

Table III.—Monkey No. 56. Mosquito Experiments (continued up to the end of October).

Mosquitoes fed on patients.		Mosquitoes fed on monkey.																						Number of times each mosquito fed on monkey.
Date.	No. of mosquitoes.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
1904.																								
Sept. 4	3
" 16	3
" 22	4
" 23	4
" 25	3
" 26	4
Oct. 10	6
" 19	4
" 24	4

Monkey No. 56 was bitten 101 times by presumably infected mosquitoes. Its serum was repeatedly tested as to agglutination of the *M. melitensis*, but no signs of a reaction were observed.

The want of success, which has up to the present attended our efforts to transfer by means of mosquitoes the *M. melitensis* from infected human beings to healthy monkeys, is disappointing but does not necessarily invalidate the result obtained by Dr. Zammit. The case upon which he made his successful experiment was unusually severe, and since then cases of this type have not been met with either in the military or in the civil hospitals.

Conclusions drawn as to the Mode of Entrance of the M. melitensis into the Body.

There is experimental evidence to show that the *M. melitensis* when present in dry dust is capable of being absorbed by monkeys.

The path of absorption may be through the nares, throat, respiratory passages, and alimentary canal. When present in food it is also taken into the system of monkeys; here, again, the path of absorption may be through the throat as well as through the mucous membrane of the alimentary canal.

When transmitted through an unbroken mucous membrane the process of absorption is comparatively slow, and under these conditions the wave of fever appears to be prolonged. The long and variable incubation period observed in monkeys infected through an unbroken mucous membrane is frequently observed in man infected under natural conditions.

When the *M. melitensis* is absorbed through a crack in a mucous membrane or in the skin, or is injected subcutaneously, the absorption is rapid and the incubation period in monkeys varies from 5 to 7 days. The curve of fever is characterised by a rapid rise usually followed by a rapid fall. These acute infections have also been observed in man infected under the same conditions, but the period of incubation appears to be longer in man than in the monkey.

The history of Monkeys Nos. 69 and 47 shows that healthy monkeys may become infected by urine secreted by monkeys suffering from Mediterranean Fever. Just as in the case of man, the *M. melitensis* is excreted in the urine of infected monkeys. And it seems probable that healthy monkeys walking in the infected secretion convey the specific microbe into the mouth by means of the paws.

Infection by means of urine secreted by cases of Mediterranean Fever readily explains the cases of Mediterranean Fever which appear to arise spontaneously in hospitals. In the absence of specific knowledge as to the mode of excretion of the *M. melitensis* from the human body, sufficient care has hitherto not been taken to sterilise bed-pans, urine bottles and sheets soiled by cases of Mediterranean Fever.

There is no evidence that Mediterranean Fever can be contracted by contact with cutaneous surfaces, uncontaminated by urine.

The experiments made with *Stegomyia fasciata* do not support the result obtained by Dr. Zammit.

5.

DESCRIPTION OF A METHOD OF CULTIVATING THE *MICROCOCCUS MELITENSIS* FROM SMALL QUANTITIES OF PERIPHERAL BLOOD AND INOCULATION EXPERI- MENTS WITH THE MICRO-ORGANISMS ISOLATED.

By Staff-Surgeon R. T. GILMOUR, R.N., Bighi Hospital, Malta.

[*Note.*—This work was kindly undertaken by Staff-Surgeon Gilmour, R.N., at the laboratory of the Naval Hospital, Malta. He has already published a paper on the subject entitled "A few Notes on the Bacteriology and Pathology of Mediterranean Fever," published in 'Health of the Navy' for 1902. In that paper he gives the result of the examination of sixteen cases of Mediterranean Fever. Out of these sixteen cases the *M. melitensis* was isolated from eight, three gave no growth, and five were uncertain as they were contaminated. In these first experiments Staff-Surgeon Gilmour used fairly large quantities of blood and incubated the blood in a large volume of broth. From 0.5—8.8 c.c. blood in from 15—60 c.c. of broth were used.—ED.]

Preparation of the Patient.

The arm should be chosen in which the veins at the bend of the elbow are the most prominent. The selected limb should be shaved from the middle of the arm to the middle of the forearm. This area should then be washed with hot sterile water, carbolic soap, and a sterile nail-brush for 20 minutes; then swabbed with ether for 10 minutes, to dissolve out the fat, and finally scrubbed with a 1 in 500 solution of perchloride of mercury for $\frac{1}{4}$ hour. A sterile dressing should then be applied, soaked in the same disinfectant, until the time of the operation, about 24 hours afterwards.

The Apparatus Required.

1. A sterile bandage.
2. A sterile 10 c.c. serum syringe.

3. (a) One flask, containing 30 c.c. of broth.
- (b) Two tubes, each containing 9 c.c. of broth.*
- (c) Sufficient Petri's dishes, each containing 10 c.c. of agar-agar.
4. A spirit lamp.
5. Sterile 1 c.c. pipettes and glass rods.
6. Six tubes, each containing 10 c.c. of broth.

Method of Extracting the Blood.

1. Remove the bandage from the dressing.
2. Constrict the arm above the elbow-joint with the sterile bandage.
3. After waiting a few minutes, so that the veins may become engorged, insert the needle into the most prominent vein and withdraw sufficient blood, about 5 c.c.
4. An assistant, holding the flask and the tubes on the slant, should then remove the plugs with sterile forceps, and the required quantities of blood (2 c.c. for the flask, and 1 c.c. for the two 9 c.c. tubes) should be passed into the broth.

The assistant should then keep the broth in the tubes well agitated, so as to prevent coagulation and get a good emulsion.

0.5 c.c. of blood should then be passed into each of the Petri dishes, and immediately spread out with a sterile rod.†

5. The next part of the procedure must be performed in the laboratory. Pass the following quantities of emulsion, from one of the 9 c.c. tubes, into others containing 10 c.c. of broth :—‡

0.1 c.c. of emulsion	=	(0.01 c.c. of blood)	into the 1st tube.
0.25 "	"	= (0.025 " ")	" 2nd "
0.5 "	"	= (0.05 " ")	" 3rd "
1.0 "	"	= (0.1 " ")	" 4th "
2.0 "	"	= (0.2 " ")	" 5th "
3.0 "	"	= (0.3 " ")	" 6th "

6. Incubate the broth tubes and Petri's dishes at 35° C., and examine daily. From the 4th to 10th day of incubation inoculate sloped agar tubes from the broths, allowing 15 drops to flow over the surface of each. Ring all colonies daily, which appear in the Petri dishes, and number them, keeping a tally of the day they appeared. From the 4th to the 10th day remove the colonies with a sterile loop, plant on agar, and incubate at 35° C.

The following are the tests applied to ascertain whether a growth is *M. melitensis* :—

* Tubes containing 19 c.c. of broth were afterwards used.

† These dishes were afterwards inoculated with 1 c.c. of 1—10 emulsion.

‡ Smaller quantities of blood were afterwards used.

1. An emulsion in normal saline is examined under the microscope.
2. Specimens are stained with Neelson's carbol-fuchsin (1 in 10).
3. Specimens are stained by Gram's method.
4. The growth is tested for agglutination with the sera of Mediterranean fever cases; controls being made with healthy serum.

The reaction of all media used in the experiments is +10A (Eyre's scale) unless otherwise stated.

Experiment I.

Harry Chapman, 28. Admitted into hospital on April 2, 1904. On June 23, 1904, the 84th day of illness, 8.0 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
4.0 c.c.	30 c.c.	Pure culture of <i>M. melitensis</i> .
1.0 "	10 "	" "
1.0 "	9 "	Used for inoculating the following tubes.
0.01 "	10 "	Negative.
0.025 "	10 "	"
0.5 "	10 "	"
0.1 "	10 "	"
0.2 "	10 "	"
0.3 "	10 "	"

Result of Inoculations of Blood on to Sloped Agar Tubes.

Amount of blood used.	Amount of medium used.	Result.
0.5 c.c.	10 c.c.	One colony of <i>M. melitensis</i> .
"	"	Three colonies of <i>M. melitensis</i> .
"	"	Sterile.

Experiment II.

J. S. Ward, 24. Admitted into hospital on June 8, 1904. On June 25, 1904, the 17th day of illness, 5.0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
2.0 c.c.	30 c.c.	Contaminated.
1.0 "	9 "	The <i>M. melitensis</i> obtained.
0.01 "	10 "	Sterile.
0.025 "	10 "	"
0.05 "	10 "	"
0.1 "	10 "	"
0.2 "	10 "	The <i>M. melitensis</i> obtained.
0.3 "	10 "	Sterile.

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0.5 c.c.	10 c.c.	Five colonies of <i>M. melitensis</i> obtained.
0.25 "	10 "	Contaminated.

Experiment III.

Alfred Law, 20. Admitted into hospital on June 20, 1904. On June 28, 1904, the 14th day of illness, 5.0 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
2.0 c.c.	30 c.c.	Contaminated.
0.01 "	10 "	Sterile.
0.025 "	10 "	<i>M. melitensis</i> obtained.
0.5 "	10 "	" "
0.1 "	10 "	" "
0.2 "	10 "	" "
0.3 "	10 "	" "
0.01 "	10 "	" "
0.025 "	10 "	" "
0.05 "	10 "	" "
0.1 "	10 "	" "
0.2 "	10 "	Broth contaminated.
0.3 "	10 "	" "

Experiment IV.

John Waters, 23. Admitted into hospital on June 29, 1904. On July 5, 1904, the 25th day of illness, 8.0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
2.0 c.c.	30 c.c.	The <i>M. melitensis</i> obtained.
1.0 "	9 "	Sterile.
0.01 "	10 "	"
0.025 "	10 "	"
0.05 "	10 "	"
0.1 "	10 "	"
0.2 "	10 "	"
0.3 "	10 "	"

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0.5 c.c.	10 c.c.	One colony of <i>M. melitensis</i> .
"	"	" "
"	"	Contaminated.
"	"	Two small contaminations. No <i>M. melitensis</i> .
"	"	One small contamination. No <i>M. melitensis</i> .

Experiment V.

Thomas Eccles, 23. Admitted into hospital on July 9, 1904. On July 19, 1904, the 23rd day of illness, 2.0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
0.01 c.c.	10 c.c.	Contaminated.
0.025 "	"	"
0.05 "	"	"
0.2 "	"	"
0.2 "	"	"

Result of Inoculations of Blood on to Sloped Agar Tubes.

Amount of blood used.	Amount of medium used.	Result.
0.1 c.c.	10 c.c.	Contaminated.
"	"	"
"	"	"
"	"	"
"	"	"

The whole of these growths were contaminated with a staphylococcus.

Experiment VI.

Edward Stedman, 32. Admitted into hospital on July 7, 1904. On July 20, 1904, the 25th day of illness, 3·5 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	One colony of <i>M. melitensis</i> .
"	"	Sterile.
"	"	Contaminated.
"	"	Sterile.

Experiment VII.

Sidney Fleetwood, 23. Admitted into hospital on June 11, 1904. On July 21, 1904, the 40th day of illness, 4·0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
1·0 c.c.	50 c.c.	Pure culture of <i>M. melitensis</i> .
0·01 "	10 "	Sterile.
0·025 "	10 "	"
0·05 "	10 "	"
0·1 "	10 "	"
0·2 "	10 "	"
0·3 "	10 "	"

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	Sterile.
"	"	"
"	"	"
"	"	"
"	"	One colony of <i>M. melitensis</i> .

Experiment VIII.

James Slater, 21. Admitted into hospital on July 13, 1904. On July 22, 1904, the 20th day of illness, 3·5 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
1.5 c.c.	30 c.c.	Pure culture of <i>M. melitensis</i> .
0.005 "	10 "	Sterile.
0.0125 "	10 "	"
0.025 "	10 "	"
0.05 "	10 "	"
0.1 "	10 "	"
0.15 "	10 "	"

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0.1 c.c.	10 c.c.	Sterile.
"	"	Contaminated.
"	"	Sterile.
"	"	Contaminated.
"	"	"
"	"	Sterile.

Experiment IX.

Arthur Witte, 27. Admitted into hospital on August 9, 1904. On August 12, 1904, the 3rd day of illness, 3.5 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
1.0 c.c.	30 c.c.	Pure culture of <i>M. melitensis</i> obtained.
0.005 "	10 "	Sterile.
0.0125 "	10 "	"
0.025 "	10 "	"
0.05 "	10 "	"
0.1 "	10 "	"
0.15 "	10 "	"

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0.1 c.c.	10 c.c.	One colony of <i>M. melitensis</i> , and one small colony of contamina- tion.
"	"	One small colony of contamina- tion.
"	"	Sterile.
"	"	Contaminated.
"	"	"
"	"	"

Experiment X.

Arthur Witte, 27. Admitted into hospital on August 9, 1904. On August 19, 1904, the 10th day of illness, 2.5 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
0.005 c.c.	10 c.c.	Sterile.
0.0125 "	"	Contaminated.
0.025 "	"	"
0.05 "	"	"

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0.1 c.c.	10 c.c.	Sterile.
"	"	One small colony of contamina- tion.
"	"	Contaminated.

Experiment XI.

Frank Murch, 26. Admitted into hospital on August 14, 1904. On August 19, 1904, the 5th day of illness, 1.0 c.c. of blood was withdrawn from the left median-basilic vein.

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0.1 c.c.	10 c.c.	31 colonies of <i>M. melitensis</i> .
"	"	33 " "
"	"	31 " "

Experiment XII.

Edward Freak, 21. Admitted into hospital on August 18, 1904. On August 22, 1904, the 24th day of illness, 1·0 c.c. of blood was withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
0·005 c.c.	10 c.c.	Sterile.
0·0125 "	"	"
0·025 "	"	Contaminated.
0·05 "	"	Sterile.
0·1 "	"	"

Experiment XIII.

Frank Murch, 26. Admitted into hospital on August 14, 1904. On August 27, 1904, the 13th day of illness, 3·0 c.c. of blood were withdrawn from the right median-cephalic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
0·0025 c.c.	10 c.c.	Sterile.
0·005 "	"	"
0·0125 "	"	"
0·025 "	"	"
0·05 "	"	"

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	Sterile.
"	"	"
"	"	Contaminated.

On August 19, 1904, this man's blood had given 316 micrococci per cubic centimetre, *vide* Experiment XI.

Table showing the Average Number of *M. melitensis* per cubic centimetres of Blood and the Day of Disease.

Experiment.	Day of disease.	Number of micrococci per cubic centimetres of blood.
I	84	2·6
II	17	10
III	14	100
IV	25	1·0
V	23	0·0
VI	25	3·3
VII	40	2·0
VIII	20	0·6
IX	3	3·3
X	10	0·0
XI	5	316·6
XII	24	0·0
XIII	13	0·0

[*Remarks.*—It is evident from Staff-Surgeon Gilmour's experiments that the *M. melitensis* is present in the majority of the cases examined. Their number is, however, so small that it seems extremely doubtful if this disease can be carried by biting insects.—ED.]

INOCULATION EXPERIMENTS ON MONKEYS WITH MICRO-ORGANISMS,
SUPPOSED TO BE *M. melitensis*, FROM THE BLOOD OF PATIENTS,
SUFFERING FROM MEDITERRANEAN FEVER.

Experiment I.

A small, healthy, female Rangoon monkey, which had been under observation for 20 days. It had gone up in weight $\frac{1}{2}$ lb., its coat had improved, and it appeared in perfect health. The temperature varied between 99°·6 and 101°·8. Its serum did not agglutinate *M. melitensis* in a dilution of 1—10. Weight 4 lbs. 12 ozs.

The object of this experiment was to prove that the coccus, obtained from the peripheral blood of a patient (W. A., age 32), was the *M. melitensis*.

October 6, 1903. This monkey was inoculated between the shoulder blades with an emulsion made from the contents of two sloped agar tubes (third generation of micrococcus) in 1 c.c. of broth.

October 7, 1903. Weight 4 lbs. 12 ozs.; appears well.

October 8, 1903. Weight 4 lbs. 10 ozs.; eating well.

October 9, 1903. Weight 4 lbs. 10 ozs.; seedy.

October 11, 1903. Weight 4 lbs. 5 ozs.; irritable, in other respects appears well. Its serum gives an immediate reaction to *M. melitensis* 1—10, 1—50, and 1—100 after 24 hours.

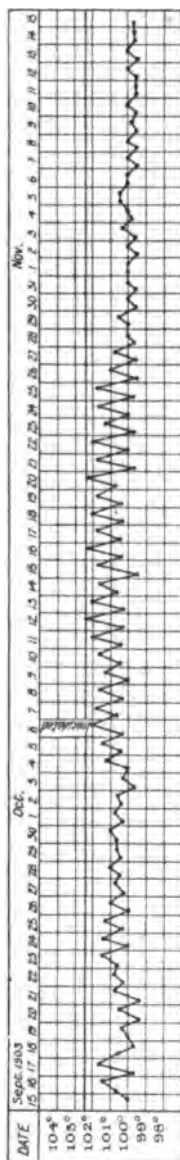
October 15, 1903. Weight 4 lbs. 2 ozs. ; good reaction 1—100 ; seedy, but not very ill.

October 20, 1903. The monkey is improving in health. Slight reaction 1—50 ; good reaction, 1—30.

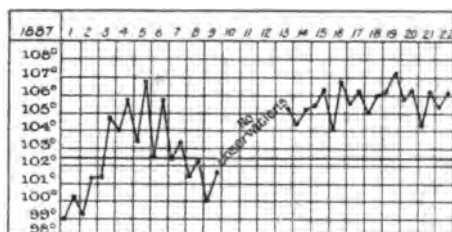
November 1, 1903. The animal has regained its weight and now weighs 4 lbs. 12 ozs. Perfectly well ; reaction 1—10.

June 10, 1904. This monkey still reacts 1—10. It had no relapse.

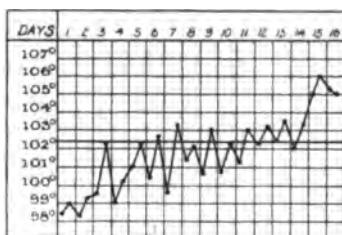
The following chart represents the temperature curve. Taken in the axilla.



[Remarks.—The temperature seems to have been taken in the axilla. It ought, in my opinion, to be taken in the rectum, the thermometer should be introduced as far into the intestine as possible, and a minimum of 5 minutes used for the observation. It is difficult to believe that this monkey can have had Malta Fever. The temperature chart shows no signs of the disease. Compare the following charts :—

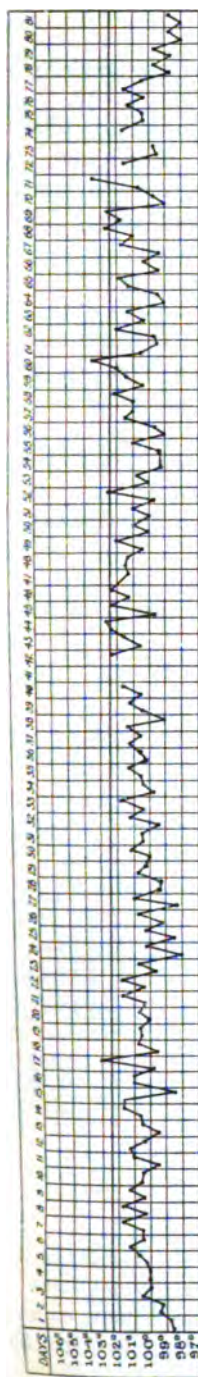


Monkey ♂. *Macacus rhesus*. Bruce. Temperature taken in the Axilla.
Growth from Spleen.

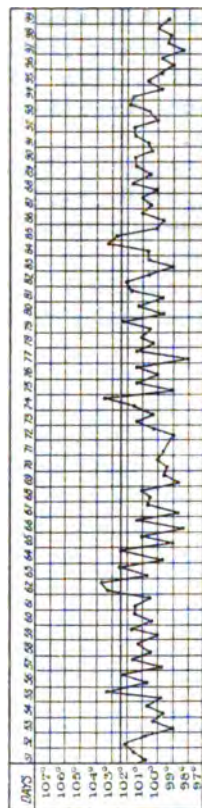
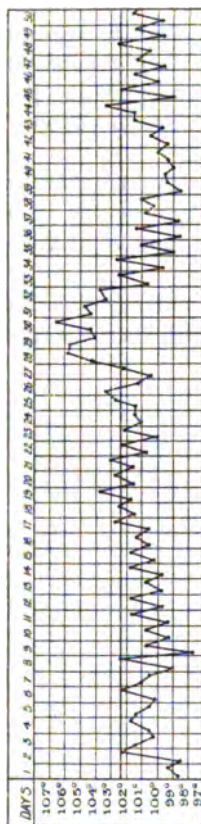


Monkey ♂. *M. rhesus*. Hughes. Axilla Temperature. Growth from Spleen.

Compare also the charts, Experiments V, VI, and XI Horrocks. In these cases the *M. melitensis* was recovered from the spleen after death. All these charts show a definite febrile disturbance, which is almost absent in the chart under consideration. It is certainly desirable that in these cases, the animal should be killed and the *M. melitensis* looked for in the spleen. Of course there is always the danger that the taking of the animal's temperature is entrusted to an ignorant or untrustworthy assistant.—Ed.]



Monkey ♂. Hughes. Axilla. Growth from Heart's Blood of Monkey.



Monkey ♀. *M. rheus*. Hughes. Axilla. Growth from Spleen of Monkey.

Experiment II.

A small, healthy, male monkey, which had been kept under observation for 28 days. Weight 4 lbs. 9 ozs. No reaction 1—10.

November 16, 1903. This monkey was inoculated into the extensor muscles of the left thigh with 1 c.c. of an emulsion, made from three tubes of *M. melitensis* (first generation) in 2 c.c. of broth.

This experiment was carried out to prove that the growth, obtained from the peripheral blood of G. F., was the *M. melitensis*.

November 24, 1903. The monkey appears perfectly well. Weight 4 lbs. 8 ozs. Immediate agglutination reaction 1—400.

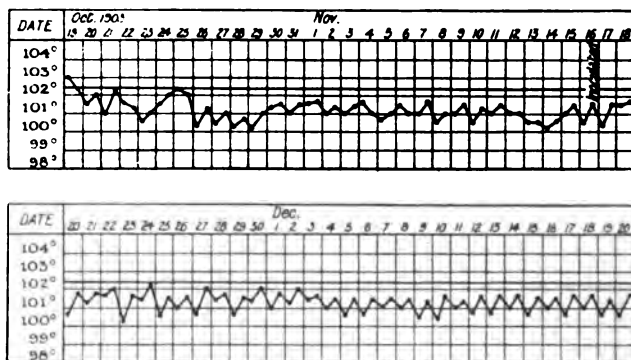
November 27, 1903. Weight 4 lbs. 8 ozs. Immediate agglutination reaction 1—400. The monkey was given a second injection of the contents of one tube, from same patient, into the muscles of the right thigh.

December 8, 1903. The monkey has remained perfectly well. Weight 4 lbs. 8 ozs. Agglutination reaction 1—200.

January 3, 1904. Monkey in good health. Agglutination reaction 1—200.

June 10, 1904. This monkey still reacts 1—10.

The following chart represents the temperature, taken in the axilla :



[Remarks.—This is also a very unsatisfactory temperature chart. The high agglutination reaction is, however, a strong argument that Staff-Surgeon Gilmour is dealing with *M. melitensis*.—ED.]

Experiment III.

The following experiment was carried out to prove that the coccus, obtained from the knee-joint of F. B., age 21, was the *M. melitensis*.

December 5, 1903. A male monkey, which had been under observation for a week, was inoculated into the extensor muscles of the left

thigh with an emulsion made from one tube (fourth generation) in 1 c.c. of sterile broth. It weighed 7 lbs. 6 ozs.; its serum would not agglutinate the laboratory *M. melitensis*; and its temperature was steady, 100° F.—100°·6 F.

The monkey remained well until December 8, the 3rd day after inoculation, when it shivered a good deal, went off its feed, and suffered from a rise of temperature, 102° F., in the evening.

After this date the monkey became very sick; its serum gave a negative reaction 1—10 on December 8; reacted 1—1200 on December 13, 1—1200 on the 17, and 1—3000 on the 20, the 15th day after inoculation; its weight decreased 1 lb. 4 ozs. by December 22, and its temperature remained up after the 3rd day, ranging between 101° and 102°·8 F.

December 23, 1903. The monkey was killed with chloroform, and a *post-mortem* held.

The organs were healthy, with the exception of the liver and spleen, which were congested. There were no signs of tubercle. Two sloped agar and two broth tubes were inoculated from the liver, three agar and two broth from the spleen, two agar from the heart's blood, and 30 c.c. of broth with 1 c.c. of heart's blood.

December 29, 1903. The tubes from the liver remained sterile and were destroyed.

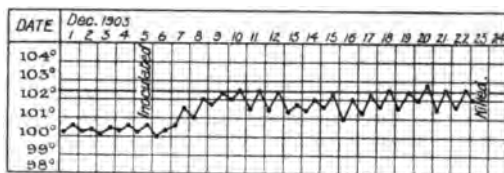
The agar tubes from the spleen showed no growth until the 3rd day, December 26, when many small isolated colonies appeared which, by the 4th day, had the appearance of a growth of *M. melitensis*. One broth tube from the same organ gave a growth by the 5th day; the other was sterile. A few transparent, isolated colonies also appeared on one agar tube from heart's blood on the 4th day.

The broth inoculated with heart's blood remained clear until the 3rd day, when it became slightly cloudy, after which the coccus grew rapidly; each field under the microscope being full of cocci. Sloped agar tubes (1 and 2), inoculated from the blood broth on December 24 and 27 respectively, remained sterile and were destroyed on December 29 and January 2. Two other tubes (3 and 4), inoculated on December 28, showed growth on December 31—isolated, transparent colonies, which the next day had every appearance of *M. melitensis*.

The tubes from the blood and spleen were examined microscopically, etc., and the growth—a micrococcus—was found to be identical in size, shape, motility, and staining reactions with the laboratory *M. melitensis*; it also gave an immediate agglutination reaction with the sera of the following Mediterranean fever patients: A. 1—500, B. 1—1000, P. 1—100, but not with healthy serum.

I think that the above experiments prove conclusively that the coccus, obtained in the first place from the synovial fluid of the knee-joint, was the *M. melitensis*.

The following chart shows the temperature curve :



[Remarks.—There can be little doubt that in this case Staff-Surgeon Gilmour is dealing with the *M. melitensis*. There is a distinct rise of temperature and the micro-organism was recovered from the spleen and blood.—ED.]

6.

ISOLATION OF THE *MICROCOCOCCUS MELITENSIS* FROM THE BLOOD.

By Dr. T. ZAMMIT, Member Mediterranean Fever Commission.

The patients of the Civil Central Hospital furnished, for the most part, the material for this investigation. The Honourable the Comptroller of the Charitable Institutions and the Medical Officers connected with that hospital deserve the thanks of the Commission for having kindly allowed the investigation to be conducted in the hospital.

The method followed at first was the simple one of drawing blood with a syringe from a vein at the bend of the arm. One to five cubic centimetres of blood was drawn, with all necessary precautions, and diluted in broth in the proportion of 1 of blood to 19 of broth. A proportion of 1 to 9 of broth was tried, but found unsuitable.

As soon as the blood was mixed with the broth it was taken to the laboratory where it was put in various proportions in 10 c.c. broth tubes and incubated. From the first mixture of blood 1, 2, 3, 4, 5 c.c., etc., were added to broth tubes and the dilution noted.

After an incubation of 4—5 days, a loopful of broth was passed over a sloped agar tube. When after 5 days no growth appeared on the agar, the same tube was reinoculated from the corresponding tube of broth, and so on every 5 days up to 1 month.

If a growth appeared having the appearance of the *M. melitensis* a note was made and the tube set aside for identification; if numerous

foreign growths appeared, the tube was usually thrown away and a note made that it was contaminated.

In some cases, however, the *M. melitensis* could be easily recognised among a lot of contaminations, and then sub-cultures were made to get a pure culture of the Micrococcus.

The contaminations observed during this investigation were traced to the imperfect preparation of the skin before drawing the blood, and, in fact, the contaminations were reduced to a minimum when a pad with carbolic solution (5 per cent.) was kept on the part for a few hours previous to the operation.

No bad effects were ever observed after the puncture, and no complaints were ever made by the patients.

After some time a few cases were met with in which, owing either to the prostrate condition of the patient or to his excessive nervousness the drawing of the blood from a vein by means of a syringe was not found to be possible. I, therefore, devised the following method of taking the blood which has proved so successful that I resorted to it constantly afterwards :—

The finger or the lobe of the ear of the patient is washed well with ether, ether-soap, water, alcohol and ether, and on the dry skin a puncture is made with a small syringe needle. With a sterile cotton wool pad the first drop of blood is removed, and an assistant squeezes the part for the next drop at the request of the operator. In a test-tube a large number of capillary tubes 1 cm. long are sterilised by dry heat, and at the time of collecting the blood, one of these short tubes is taken with fine forceps passed, immediately before, through the flame. As soon as the assistant squeezes the part and removes the cotton-wool pad, the tube is brought in contact with the drop, and when full is immediately put in a broth tube. This operation is repeated as long as the blood continues to ooze; six tubes are usually filled. From these broth tubes, marked and incubated, passages on agar are made in the usual manner.

When a growth of *M. melitensis* is obtained on the agar slope, the capillary tube is drawn out of the broth, washed, dried, and weighed. It is then weighed again full of distilled water, and the difference between the two weights gives the volume of liquid the tube can hold, thus establishing to a nicety the amount of blood from which the *M. melitensis* has been isolated. By this method a volume of 0.005 of 1 c.c. of blood has been easily and accurately measured.

This method was used in twenty-two cases out of fifty with good results. Greater care is, of course, required in the disinfection of the skin, but when this extra trouble is taken the results compare most favourably with the bleeding from a vein. This method has also the great advantage that it can be applied to animals, as in case No. 50 in Table A.

Table A.

Order number.	Name and surname.	Sex.	Age.	Date of illness.	Character of case.	Temperature of body at time of experiment.	Amount of blood taken.	Minimum amount of blood in which <i>M. melitensis</i> was found.	Date of observation.	Remarks.
				Day.			c.c.	c.c.	1904.	
1	Giorgio Abdilla	M	40	25	Mild	99.8	2	—	June 21	No growth whatever.
2	Paolo Spiteri	M	49	100	"	99.0	1	—	"	"
3	Emmanuele Caruana	M	24	65	"	99.8	1	0.1	June 23	"
4	Ursola Vassallo	F	56	20	"	101.0	1	0.1	"	"
5	Ursola Vassallo	F	56	20	"	101.0	1	0.1	June 27	"
6	Salvatore Camilleri	F	28	120	"	100.2	1	0.2	"	"
7	Maria Chelcuti	F	29	13	Acute	100.0	1	0.1	July 7	"
8	Carmela Dimech	F	18	35	Mild	101.0	1	0.1	"	"
9	Giuseppe Cordina	M	31	240	"	99.8	1	—	July 8	"
10	Alfredo Scicluna	M	38	14	Acute	102.0	1	0.1	"	"
11	Pasquale Cachia	M	33	8	Mild	99.0	1	—	July 11	"
12	Francesco Saliba	M	45	15	Acute	99.0	1	0.1	"	"
13	Salvatore Ungaro	M	25	30	Mild	100.0	1	—	July 15	"
14	Antonina Hili	F	23	8	Acute	104.4	few drops	0.02	"	Tubes contaminated.
15	Luigia Brina	F	45	18	"	103.2	1	—	"	No growth whatever.
16	Carmelo Camilleri	M	21	30	Mild	103.2	1	—	"	"
17	Giuseppe Farrugia	M	36	14	"	101.0	1	0.1	July 18	"
18	Mosè Azopardi	M	29	10	Acute	103.4	1	0.1	"	"
19	Luigia Brina	F	45	16	"	103.4	1	0.1	July 22	"
20	Raffaele Mercieca	M	15	7	"	105.4	1	0.1	"	Tubes contaminated.
21	Carmelo Fava	M	24	13	Mild	102.4	1	—	"	"
22	Simcone Cumbo	M	30	15	"	102.4	1	0.2	"	"
23	Giuseppe Micallef	M	39	18	"	102.4	1	0.1	"	"
	Caterina Pons	F	44	54	"	102.0	1	0.1	July 27	"

24	Angelo Inguanez.....	M	37	12	Acute	102.0	1	0.5	"		
25	Nattar Bessar	M	22	7	Mild	101.0	1	0.1	"		
26	Marianne Grima	F	24	82	"	101.0	1	0.1	"		
27	Vincenzo Mamò	M	27	22	"	101.0	few drops	—	"		
28	Salvatore Bonanno	M	46	18	"	101.0	"	—	"		
29	Carmelo Vella	M	26	150	"	99.0	"	—	"		
30	Patrick Bourke	M	33	65	"	99.4	"	—	"		
31	Giovanni Buhagiar	M	29	6	Acute	102.0	"	—	Aug. 4		
32	Carmelo Micallef	M	15	33	Mild	99.0	"	0.0097	Aug. 9		
33	Giuseppe Zammit	F	17	7	Acute	104.0	"	0.1	Aug. 10		
34	Gio. Maria Miffend	M	55	10	"	101.0	1	0.025	Aug. 11	No growth whatever.	
35	Carmela Zammit	F	17	8	"	103.8	1	—	Aug. 11	"	
36	Maria Teresa Perini	F	22	21	Mild	102.0	few drops	—	Aug. 11	No growth whatever.	
37	Maria Anna Fenech	F	25	30	"	102.0	"	—	Aug. 12	"	
38	Nicola Farrugia	M	49	18	"	100.6	1	0.025	Aug. 12	"	
39	Taccredi Piacentini	M	31	7	Acute	102.2	1	0.05	"		
40	Carmelo Grech	M	43	35	Mild	102.4	1	0.005	Aug. 17		
41	S. Valder	F	25	7	Acute	104.0	few drops	0.009	Aug. 17		
42	Carmela Bugeja	F	27	17	"	105.0	"	0.008	"		
43	Giuseppa Grima	F	43	60	"	106.0	"	—	Aug. 22	Tubes contaminated.	
44	Anna Zammit	F	40	120	Mild	103.0	"	—	Aug. 24	"	
45	Jos. Sullivan	M	6	14	Acute	102.4	"	—	Aug. 25	"	
46	Carmelo Delicata	M	56	18	Mild	99.0	"	—	"	No growth whatever.	
47	Vincenzo Abela	M	32	8	"	101.0	"	0.006	"	"	
48	Gaetano Biliion	M	21	6	Acute	103.0	"	—	"	"	
49	Nicola Farrugia	M	48	31	Mild	103.0	"	0.005	Aug. 27	"	
50	Monkey No. 63	M	—	16	Acute	105.0	"	—	"	"	

The examination of fifty cases, made between June 21 and August 27, show that the *M. melitensis* circulates freely in the blood during an attack of fever, and that the amount of Micrococci varies usually with the temperature of the body.

In the fifty cases tabulated the *M. melitensis* was never recovered when the body temperature was below 100° F. At 102° and over it was recovered with the exception of two cases (Nos. 36 and 37), in which the tubes remained sterile, and in four cases in which the tubes were hopelessly contaminated. From one of these cases (No. 15) the *M. melitensis* was isolated by one of my colleagues on the same day.

Attempt to infect a monkey by means of a mosquito which had previously fed on a Mediterranean Fever patient.

Several mosquitoes (*Stegomyia fasciata*), which had previously been fed on an infected monkey (No. 45), were made to bite two healthy monkeys. No positive results were obtained. A positive result was obtained on the third attempt.

The third monkey (No. 63) was bought in Malta, along with two others, from a ship coming from the East Indies. Its temperature was taken twice daily after July 18, and it kept always within normal limits up to August 15.

The monkey was kept on the terrace on a side facing south-east, along with seven other animals, none of which had ever been ill.

On July 27 the blood of this monkey was tested, and it did not react to *M. melitensis* when diluted to 1 in 10.

On August 10 at 11 A.M. the monkey was bitten by two *Stegomyias* which had been fed at 11 A.M. on August 8 on a patient affected with a sharp relapse of Mediterranean Fever at the Civil Hospital (patient P. Sillato, Bed No. 40).

On August 20 the monkey was bitten again by one of the two *Stegomyias* used on the 10th.

On August 23 (13 days after inoculation) a rise of temperature was observed, and the blood of the animal was tested for Mediterranean Fever reaction, but no clear reaction could be obtained.

On August 26 the temperature rose again, and on the blood being tested, it was observed that it reacted strongly to *M. melitensis*. An immediate and complete agglutination was obtained at various dilutions up to 1 in 300. No further dilutions were tried.

The animal had obviously a sharp attack of fever, but the isolation of the coccus from the blood was necessary to make sure of the disease.

Without killing the animal, on August 31 one of its ears was properly disinfected and blood was drawn by pricking a small vein. The

blood was collected in small capillary pipettes 1 cm. long, in the manner described in another part of the Report, and put in broth.

On September 1 passages on agar were made from the broth tubes, and on the 4th a distinct growth was observed in one of the tubes. On the 5th two other tubes were found to have grown the Coccus.

All the growths tested in the ordinary way showed that the microbe was the *M. melitensis* in pure culture.

The least amount of blood from which the *M. melitensis* was obtained in this case was 0.005 c.c. Smaller quantities were not tried.

The position of the other monkeys, both healthy and ill, at the time of the experiment, is shown in the plan (p. 42). It is easily seen that no infected monkeys were anywhere near No. 63, and, therefore, direct infection from the monkeys, then ill on the same terrace, is highly improbable.

EXPERIMENTS MADE IN MALTA BY DR. ZAMMIT BEFORE THE APPOINTMENT OF THE COMMISSION.

1. To Test Vitality of *M. melitensis* on Filter-paper exposed to Diffused Light.

August	27, 1903.	A strip of filter-paper was hung on a wire inside a test-tube plugged with cotton-wool and sterilised by dry heat.			
"	28, "	Strip of filter-paper smeared with loopful of agar culture. Twelve tubes prepared in the same manner.			
September 1,	"	The filter-paper dropped in a broth tube and incubated. Growth obtained in due time.			
"	2, "	"	"	Same result.	
"	3, "	"	"	"	
"	4, "	"	"	No growth obtained.	
"	5, "	"	"	"	"
"	6, "	"	"	"	"

Conclusion.—*M. melitensis* retained its vitality for 7 days in diffused light. This experiment was repeated three times with the same result.

2. To Test Vitality of *M. melitensis* in various Coloured Lights.

Agar tubes inoculated with a drop of broth culture were incubated in cardboard boxes, of which the cover was made of a coloured glass plate. Violet, red, green, yellow, and blue plates were used. One tube was left in diffused light, and another one was wrapped in black paper.

Result.—No difference in growth was observed in the different tubes. The experiment was repeated three times with the same result, the tube exposed to blue light showing once a richer growth than the rest.

3. Action of Direct Sunlight on Growth of *M. melitensis* in Agar Tubes.

September 17, 1903. Agar tube inoculated with 1 drop of broth culture was exposed for 15 minutes to the direct action of sunlight at about noon. Control tubes left in diffused light. No growth appeared before the 3rd day, but on the 4th day a growth was seen which in a few days was much more luxuriant than that on control tubes.

The experiment was repeated twice with the same result.

4. Vitality of *M. melitensis* on Ordinary Limestone.

September 12, 1903. Small bits of ordinary white porous limestone were taken and thoroughly sterilised. Emulsion made of *M. melitensis* from agar in sterile distilled water and the bits of stone wetted with this. The whole was kept in a dry atmosphere. On the 3rd day bits of the stone were dropped in broth tubes.

As former experiments had shown that light favours the growth of the *M. melitensis*, part of the bits of stone wetted with *M. melitensis* emulsion was kept in diffused light and part in a tube wrapped in thick black paper. The other conditions of the two tubes with pieces of stone were the same.

The result of the experiment was as follows:—

	Stone kept in dark.	Stone kept in diffused light.
Sept. 15 (3rd day).	Growth of <i>M. melitensis</i> .	Growth of <i>M. melitensis</i> .
„ 18 (6th „).	„ „	„ „
„ 19 (7th „).	„ „	„ „
„ 20 (8th „).	„ „	„ „
„ 26 (14th „).	No growth.	„ „
Oct. 28 (46th „).	„	„ „
Nov. 2 (51st „).	„	„ „
„ 19 (68th „).	„	No growth.

Conclusion.—Vitality of *M. melitensis* on limestone, in the dark, from 8 to 14 days.

Vitality of *M. melitensis* on limestone, in diffused light, not less than 51 days.

The experiment was repeated three times with practically the same result.

5. To Test the Action of *M. melitensis* on the Reaction of Media.

September 22, 1903. Seventy cubic centimetres of peptone broth with a reaction of +6, Eyre's scale, inoculated with loopful of *M. melitensis* from agar, and incubated at 37° C.

„ 26, „ Acidity reduced to +2.
October 28, „ Broth distinctly alkaline.

6. October 29, 1903. A series of test-tubes with 20 c.c. of broth in each were inoculated with a loopful of agar culture of *M. melitensis*. The tubes were then placed in large Buchner tubes half full with water and lightly covered so as to reduce the evaporation to a minimum. The whole was then incubated at 37° C. Tubes with broth were put for control in the same conditions.

November 19, „ (20th day). Acidity of broth + 2.

January 21, 1904 (82nd „). Broth alkaline - 3.

February 18, „ (110th „). „ - 4.5.

The control tubes showed an increased acidity. On the 20th day the acidity in the control tubes had doubled.

(This experiment is being repeated.)

7.

INTERIM REPORT OF EXPERIMENTAL WORK IN THE INVESTIGATION OF MEDITERRANEAN FEVER DEALING WITH BLOOD, SKIN, SWEAT, FILTRATIONS, AGGLUTINATING SERUM AND VARIOUS INOCULATIONS ON DIFFERENT ANIMALS.

By Staff-Surgeon E. A. SHAW, R.N., Member Mediterranean Fever Commission.

Examination of Blood.

The peripheral blood of Malta Fever patients has been examined by me for the *Micrococcus melitensis* (hereafter referred to as *M. melitensis*) in fifty-one cases, the results of which I append in a tabular form.

Method.—Bend of elbow prepared as for a surgical operation, blood withdrawn from median-basilic vein direct by means of carefully sterilised serum syringe.

$\frac{1}{2}$ c.c. distributed over surface of agar in a Petri dish A.				
1	„	„	„	B.
2	„	„	„	C.
1	„	put into a 19 c.c. peptone broth tube		„ D.
1	„	„	another 19 c.c.	„ E.

ABCD kept intact, E used for making dilutions immediately, first well mixing blood and broth through a series of broth tubes by means of graduated pipettes sterilised in boiling water. At first the dilutions proceeded by multiples of 10; for instance, tube D contained 1 c.c. blood and 19 c.c. broth = a dilution of $\frac{1}{20}$, $2\frac{1}{2}$ c.c. of this contained $\frac{1}{8}$ c.c. blood and added to a 10 c.c. broth tube = $\frac{1}{8}$ c.c. of blood in $12\frac{1}{2}$ of mixture = a dilution of $\frac{1}{100}$; and abstracting 1 c.c. of this ($\frac{1}{100}$ c.c. of blood) and adding to a 9 c.c. broth tube = $\frac{1}{100}$ c.c. of blood in 10 of mixture = $\frac{1}{1000}$ dilution and so on up to $\frac{1}{100000}$.

All broth tubes and plates were duly labelled with a serial number for each patient, the quantity of blood contained, and the date and placed in the incubator at 37° C.

As time went on and the series of bloods increased it was found that *M. melitensis* was only being recovered from relatively large quantities of blood, up to Blood 15 never even from $\frac{1}{100}$ c.c. of blood and only occasionally from $\frac{1}{8}$ c.c., intermediate dilutions containing $\frac{1}{2}$ c.c., $\frac{1}{4}$ c.c., and $\frac{1}{16}$ c.c. of blood were, therefore, made and incubated for Bloods 16, 17, 18, 19. The primary dilutions in Bloods 20 to 25 were made by multiples of 3 from the $\frac{1}{20}$ dilution, i.e., $\frac{1}{60}$, $\frac{1}{180}$, $\frac{1}{540}$, $\frac{1}{1620}$, and $\frac{1}{4860}$. From Blood 26 onwards to Blood 51 by multiples of 2; thus one tube containing 19 c.c. of broth and one of blood remained as the unit 1 c.c. of blood, the other tube of similar contents had 10 c.c. abstracted and was hence left containing $\frac{1}{2}$ c.c. blood, the 10 c.c. removed was added to a 10 c.c. broth tube, the resulting 20 c.c. of mixture well amalgamated, and 10 c.c. then abstracted thus leaving it containing $\frac{1}{4}$ c.c. blood; and thus tubes containing $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, and $\frac{1}{256}$ c.c. respectively of blood were prepared, the intention being to increase these dilutions if *M. melitensis* was ever recovered from the highest, though the first twenty-five bloods drawn had not yielded it in so high a dilution as $\frac{1}{100}$.

These blood dilutions were daily thoroughly well shaken to give the *M. melitensis* an opportunity of emerging from the leucocyte in which it was thought to be most probably lodged, and after 5 days incubation, subcultures on to agar slopes from the respective broth tubes were prepared and incubated at 37° C. These inoculations of agar slopes were repeated when considered necessary and no blood dilution was abandoned as unfruitful till it had been incubating at least 11 days.

The Petri dish method was worked side by side with the broth enrichment method for the first seventeen cases, afterwards it was abandoned. The original idea was that the number of colonies of *M. melitensis* appearing could be taken as an index of the quantity of *M. melitensis* in the measured quantity of blood taken. It was found quite easy by inclining the plate to get the blood put on the agar surface to spread itself quite evenly over the whole area of agar forming a very thin layer, but when, as in Cases 10, 12, 14, and 16,

M. melitensis was recovered by the broth method, while the plate method failed to show it, time was felt to be too valuable to persevere with the latter.

Of the details given in the tabulated results some explanation is necessary. In the column headed nation and sex, E = English, M = Maltese, A = Army, N = Navy, F = Female, and as the only female patients from which blood was taken were Maltese, the sex is specified only for that nationality, thus M.M. = Maltese male, and M.F. = Maltese female. The English patients were all male.

The temperatures given preceding drawing of blood are for the few days immediately prior to drawing of blood, the last being the temperature on day of abstraction of blood, these are given as follows: $\frac{10}{9}$ the upper temperature being the morning the lower the evening temperature. In some of the Maltese cases where, owing to the frequent unexpected discharge of patients at their own request prompt action was necessary, blood was taken very soon after admission, and in such cases temperature for only 1 or 2 days could be so given.

The day of disease is enumerated from the first onset of symptoms attributable to the fever.

The time at which blood was drawn is given, it was noted with the intention of seeing if any difference in result would appear between blood taken in the forenoon and that taken in the evening, the patient's temperature at time of drawing is here given also.

The agglutination test was applied by me to all samples of blood drawn, to independently confirm the diagnosis of Malta Fever, and after working out eighteen bloods, it was felt it would be of interest to know the *limit dilution* which would agglutinate a standard fresh agar growth of *M. melitensis* to see if there was any relation between amount of *M. melitensis* obtained from a given blood and the agglutinating power of the latter. The standard taken is an arbitrary one, being that agglutination should be unmistakably marked under the $\frac{2}{3}$ inch objective, 15 minutes after the mixing of *M. melitensis* emulsion and diluted serum, invariably comparison was made with a control.

In the column headed Recovery of *M. melitensis* the sign + means recovery, and the sign - means no recovery.

Smallest quantity of blood means the smallest quantity calculated from the highest broth dilution yielding *M. melitensis* and the amount of blood therein contained.

The following tests were invariably applied to each recovery of *M. melitensis* before it was entered as such in the laboratory records:—

1. Growth on agar slope should be that characteristic of *M. melitensis*.

2. Size and appearance of cocci in film stained with dilute carbolfuchsin should be characteristic.

No. of case.	Nation and sex.	Age.	Stage of the fever.	Temperature of patient for few days preceding bleeding.	Day of disease.	Time of bleeding and patient's temperature.	Maximum dilution of patient's blood swing aggl.	Recovery of <i>M. malleotensis</i> .	Smallest quantity of blood giving <i>M. malleotensis</i> .
1	E. A.	37	{ Had 3 waves. Now convalescent	° F. Normal for preceding 20 days	98th	12.30 noon, N.	Aggl.	+	$\frac{1}{2}$ c.c.
2	"	31	{ Had 1 wave	Normal for preceding 7 days	30th	12.30 noon, N.	Aggl.	-	-
3	"	28	{ 'T', never normal since admitted; a long severe case	E.T.'s = 101, 100, 99, 98	101st	12.30 noon, N.	Aggl.	-	-
4	"	31	{ Mild case, 4 waves ..	Normal for preceding 30 days	108th	12.30 noon, N.	Aggl.	-	-
5	M. M.	40	{ End of 4th wave ..	99 98 99 93 98'4,	74th	Noon, N.	Aggl.	-	-
6	"	22	{ In 1st wave	101'6 101 101 102'4 102'6,	15th	Noon, 100°-8	Aggl.	-	-
7	"	24	{ End of 2nd wave..	99'4 99 98 98 101' 99' 99,	40th	11.30 noon, N.	Aggl.	+	$\frac{1}{10}$ c.c.
8	M. F.	56	{ End of 2nd wave..	N. N. N. N. 100' 99'6, 99,	30th	11.30 A.M., N.	Aggl.	+	$\frac{1}{10}$ c.c.
9	"	28	{ Nearing end of 2nd wave	99 99 99'6 100'4 102' 101'6, 101'2,	41st	Noon, 100°	Aggl.	-	-
10	"	18	{ No information ...	N. 99 98 101 101' 101' 101'6,	37th	11.45 A.M., 100°-6	Aggl.	+	$\frac{1}{2}$ c.c.
11	M. M.	31	{ Now in hospital for orohitis. Had fever 8 months ago	Normal for months	240th	Noon, N.	Aggl. $\frac{1}{10}$	-	-

12	"	38	In 1st wave	100 99.6 99 100 100, 101, 100, 102	10th	5.15 P.M., 102°	Aggl.	+	½ c.c.
13	"	30	In 1st wave	102.6 99	9th	5.30 P.M., 99°	Aggl.	-	
14	"	47	{ Ill at home 3 months. Now admitted because worse	100 101 100 100 102, 101, 101, 99.4	95th	5.0 P.M., 99° 4	Aggl.	+	½ c.c.
15	"	25	{ Nearing end of 1st wave	101 101 99.2 99.1 101, 99.2, 99.2	31st	5.20 P.M., N.	Aggl.	-	
16	"	22	Middle of 3rd wave	99 99 100.4 102 103.6 103.2, 103.2, 103.2, 102, 103.2	38th	5.10 P.M., 103° 2	Red	+	½ c.c.
17	"	36	In 1st wave	101.3 101 101.1 101 100.2, 101, 103, 101, 101.6	17th	5.25 P.M., 101° 6	Aggl. 7b	-	
18	"	29	In 1st wave	102.8 103.4 102.4 103 101 101.2 101	9th	5.30 P.M., 103° 4	7b	+	½ c.c.
19	M.F.	44	In 1st wave	103.6 102.4 103 103 102 100 100 100 99.6 100.2	15th	5.45 P.M., 100° 5	7.5	+	½ c.c.
20	E.N.	22	In 2nd wave	103.6 102.6 102.4 102.4 101 102.2 97.6 N. N.	22nd	10.30 A.M., 100°	7b	-	
21	"	32	In 1st wave	102.4 103 99.6 100 102.6 102	28th	10.20 A.M., N.	Red	-	
22	M.M.	15	In 1st wave	104.2 105.4 101 101	11th	5.30 P.M., 103° 8	7b	+	½ c.c.
23	"	24	{ In 1st wave. Con- tinuous fever	102.4 102.4 103 102 101.4 101.6 99.8	31st	5.40 P.M., 102°	7b	+	½ c.c.
24	"	29	In 1st wave	103 102.6 102.4 101 101.4 99.4 99.4 100 100	13th	5.50 P.M., 99° 4	Red	-	
25	M.F.	39	In 1st wave	101 101.6 101.4 101.4 102.4 100 100.8 98 101	18th	6.10 P.M., 101° 4	Red	-	
26	M.M.	37	In 1st wave	102.4 102 101 102 100	12th	5.0 P.M., 102°	Red	+	1 c.c.
27	"	22	In 1st wave	100 101	7th	5.15 P.M., 102°	7b	+	½ c.c.

No. of case.	Nation and sex.	Age.	Stage of the fever.	Temperature of patient for few days preceding bleeding.	Day of disease.	Time of bleeding and patient's temperature.	Maximum dilution of patient's blood giving aggl.	Recovery of <i>M. mellei-tensis</i> .	Smallest quantity of blood giving <i>M. mellei-tensis</i> .
28	M. F.	24	{ In 1st wave. Continuous fever	99.6, 101.2, 101, 99, 100, 102.8, 103, 101.2, 101.8, 103.2	32nd	5.30 P.M., 102°	1000	-	
29	"	44	{ In 2nd wave.....	101, 99, 100.2, 100.2, 101.4, 102.4, 102.6, 102, 101.6, 102	56th	5.45 P.M., 101°-8	1000	+	$\frac{1}{4}$ c.c.
30	E. A.	23	{ In 1st wave	101, 100.6, 100.6, 100.5, 100.4, 102.3, 102.6, 102.8, 101.8, 102	15th	11.0 A.M., 100°	1000	+	$\frac{1}{16}$ c.c.
31	"	27	{ In 1st wave	100, 101.6, 101.6, 101.6, 102, 104, 103, 103, 104.2, 102	22nd	11.15 A.M., 101°-8	1000	-	
32	"	37	{ In 1st wave	99, 99.6, 101, 102, 99.4, 101.6, 103.4, 102.6, 103, 103	36th	11.30 A.M., 99°-8	1000	+	1 c.c.
33	M. M.	55	{ In 1st wave	100, 99.4, 99.2, 99.4, 101, 100, 100.6, 100.6, 102.2, 101	10th	5.10 P.M., 101°	1000	+	1 c.c.
34	"	17	{ In 1st wave, 100.6, 100.6, 102.2, 101, 108, 101, 101, 99.2, N. 99.2, 104	8th	5.30 P.M., 106°-8	1000	+	$\frac{1}{16}$ c.c.
35	"	38	{ In 1st wave	101, 101, 101, 102, 100.6, 101, 101, 101, 101.8, 102.3	18th	5.0 P.M., 100°-6	1000	+	$\frac{1}{4}$ c.c.
36	"	31	{ In 1st wave, 103.6, 103.6, 101.8, 101.8, 101.8, 102.3, 102.3, 101.8	7th	5.10 P.M., 103°-2	1000	+	$\frac{1}{16}$ c.c.
37	"	43	{ No information, 102.4, 102.4, 102.4, 102.4, 102.4, 102.4, 102.4, 102.4, 102.4	36th	5.30 P.M., 103°-4	1000	+	$\frac{1}{16}$ c.c.
38	E. A.	23	{ Middle of 2nd wave	102.6, 102.8, 101, 102, 102.6, 103, 104, 103.6, 103.4, 102	26th	5.40 P.M., 102°	1000	+	$\frac{1}{16}$ c.c.

[illegible]

3. Non-staining with Gram.
4. No development of gas, acidity or coagulation when grown in litmus milk, but production of alkalinity.
5. No production of acidity, but production of alkalinity when grown on glucose-litmus-agar.
6. Mobility in hanging drop merely Brownian, no translation from portion to portion of field.
7. Should be agglutinated, visibly to the naked eye by a $\frac{1}{500}$ dilution of a pure animal serum, obtained by inoculating an animal (rabbit and monkey were both used), with a pure standard growth of *M. melitensis*. Comparison with a control was always made, and the two submitted to my fellow-worker, Major Horrocks, R.A.M.C., at the next bench, and unless he concurred as to the indubitable nature of the reaction it was not accepted.

There has been considerable difficulty in extending this series of blood examinations even so far as it has gone. Patients did not like it; some consented freely, others reluctantly, and their physicians were not prepossessed in favour of it either. One would have liked to have taken a few cases and taken specimens of blood every day or every other day, and so ascertained when the *M. melitensis* appeared in and disappeared from the peripheral blood during the whole course of the fever; but it was found impossible to accomplish this. Only with one patient did I succeed in getting blood twice for examination; the first time reported as No. 6, result negative; and the second time as No. 16, result positive.

As regards syringes, I found it simplest to sterilise them in the autoclave at 120° C. The needles I found did best sterilised in pure olive oil at about 140° C.; this prevented rust and their points retained their primitive sharpness. I also found blood was obtained with greater facility if the needle were passed into the vein from the bend of the elbow towards the hand, so that blood entered the syringe in the direction of natural flow.

This method of taking blood from the median basilic vein and incubating it in broth was apparently first described by Dr. Jules Courmont, at a meeting of the Société Médicale des Hôpitaux de Paris, December 27, 1901, who applied it successfully in nine cases of Typhoid Fever in which he recovered *B. Typhosus* from the peripheral blood. I saw the method in application in Vidal's Clinique in the Hôpital Cochin in Paris in the winter of 1902-3, there studied it and applied it successfully to the recovery of *M. melitensis* from the peripheral blood of Malta Fever patients in the summer of 1903. So far as I know the dilution method to determine the smallest quantity of fluid containing the micro-organism has not hitherto been applied in the recovery of micro-organisms from the circulating blood, though it is classical in the history of the bacterial analysis of water. It has

obvious advantages over the plating method, a most important one being that as in Blood No. 27, there were only nine growths representing the nine dilutions $1, \frac{1}{2}, \frac{1}{4}, \frac{1}{8}, \frac{1}{16}, \frac{1}{32}, \frac{1}{64}, \frac{1}{128}, \frac{1}{256}$ c.c., to examine and put through the various tests for *M. melitensis*; whereas had 1 c.c. of this blood been plated out, it would presumably have yielded over 200 colonies, which would have required verification individually, as unfortunately all the colonies found in a blood plate are not necessarily of the same kind, and one cannot apply the principle *Ex uno omnes disce*.

Conclusions.

1. *M. melitensis* exists in the blood of patients in relatively very small amount, the smallest quantity of blood in which it has been found, $\frac{1}{256}$ c.c. is practically the equivalent of 4 c.mm. and as 1 cubic millimetre of blood = 5,000,000 corpuscles, and if *M. melitensis* is never found in association with less number of corpuscles than 20,000,000 it is obvious there is no comparison between this and such a disease as anthrax, for instance, in which in the blood the number of bacilli has been found in some cases equalling the number of corpuscles. This has an important bearing on the question of transmission of infection by mosquitoes.

2. No definite relation can be established between any given stage of the disease and the presence of *M. melitensis* in the blood. It has been found as early as the 7th day Cases 27 and 36, and as late as the 95th and 98th day Cases 1 and 14. It has been found in the majority of cases when the temperature of the patient has been raised, but it has been also present in convalescence (Case 1), and when temperature has been normal (Cases 7, 8 and 39), for several days, but it has also not been found when the temperature was high, Cases 6, 25, 28, 31, and 48.

3. There is some indication of a diurnal variation in its presence in the blood, out of 29 cases where blood was taken in the forenoon between the hours of 10 and 12.30, it was present in 14, absent in 15. Out of 22 cases where blood was drawn in the evening between 5 and 6.30 p.m. it was present in 16, absent in 6; a ratio of almost 3:1 in favour of the evening.

4. No relation can be established between the agglutinating power of a patient's blood for *M. melitensis* and the amount of the latter present in the blood, most of the cases in which it was found had a high agglutinating power, but one of the cases in which *M. melitensis* was found in one of the smallest quantities of blood, $\frac{1}{128}$ c.c. (Case 37) only agglutinated in a $\frac{1}{16}$ dilution, as against another in which it was found in $\frac{1}{256}$ c.c., in which there was agglutination with a dilution of 1 in 1000, and others where it was not found at all where there was agglutination in a dilution of $\frac{1}{1500}$, Cases 41, 44, and 48.

5. In some of the cases the *M. melitensis* was found to have skipped some of the dilutions, for instance, in Case 34, where the dilutions proceeded by powers of 2 from 1 to 256, *M. melitensis* was found in the 1 c.c., $\frac{1}{2}$ c.c., $\frac{1}{4}$ c.c., dilutions, absent from the $\frac{1}{8}$ c.c. and $\frac{1}{16}$ c.c. dilutions, present in the $\frac{1}{32}$ and $\frac{1}{64}$ dilutions, absent in the rest. In Blood 37, in which same series of dilutions were made, *M. melitensis* was present in all up to the $\frac{1}{64}$ c.c. inclusive, with the exception of the $\frac{1}{16}$, these were the only two cases out of the fifty-one in which this jumping took place. It is certainly not due to inadequate mixing of the dilutions, for the primary blood dilution, from the moment the blood got into it, which was instantly on the needle being withdrawn from the vein, was agitated vigorously until a considerable froth was on its surface, and so on with the succeeding dilutions. It may possibly be due to the small quantity of *M. melitensis* in the blood, or to the *M. melitensis* being in some dilutions so phagocytosed as to be unable to escape and multiply.

Examination of Bloods.

Table showing in chronological order the date of the disease in each case in which blood was taken for bacteriological examination, and the result. The fractions of a cubic centimetre indicate the smallest amount of blood from which *M. melitensis* was obtained; the sign - means no *M. melitensis* was recovered; the days of disease which are not represented by a blood examination are shown blank. It will be seen that while many days are blank, others are represented by 1, 2, 3, or 4 examinations of blood. This has been unavoidable; the number of cases willing to submit to venous puncture was too small to admit of selection; and waiting a few days usually meant losing the case.

Day of disease.	Recovery and quantity or no recovery.	Day of disease.	Recovery and quantity or no recovery.	Day of disease.	Recovery and quantity or no recovery.
1		38		75	
2		39		76	
3		40		77	
4		41	—, $\frac{1}{18}$ c.c.	78	
5		42	$\frac{1}{18}$ c.c.	79	
6		43		80	
7	$\frac{1}{8}$, $\frac{1}{18}$ c.c.	44		81	
8	$\frac{1}{18}$ c.c.	45		82	
9	—, $\frac{1}{8}$, $\frac{1}{18}$ c.c.	46		83	
10	$\frac{1}{8}$, 1 c.c.	47		84	
11	$\frac{1}{8}$ c.c.	48	—	85	
12	1 c.c.	49	$\frac{2}{10}$ c.c.	86	
13	—	50		87	
14		51		88	
15	—, $\frac{1}{8}$, $\frac{1}{18}$, $\frac{1}{18}$ c.c.	52		89	
16		53		90	
17	—, —	54		91	
18	—, $\frac{1}{8}$ c.c.	55	$\frac{1}{32}$, $\frac{1}{32}$ c.c.	92	
19		56	$\frac{1}{8}$ c.c.	93	
20		57	—	94	
21		58		95	$\frac{1}{8}$ c.c.
22	—, —, 1 c.c.	59		96	
23		60		97	
24		61		98	$\frac{1}{8}$ c.c.
25	$\frac{1}{18}$ c.c.	62		99	
26	$\frac{1}{18}$ c.c.	63		100	
27		64		101	—
28	—, —	65		102	
29		66		103	
30	—, $\frac{2}{18}$ c.c.	67		104	
31	—, $\frac{1}{8}$ c.c.	68		105	
32	—	69	—	106	
33		70		107	
34	$\frac{1}{8}$ c.c.	71		108	—
35		72		240	—
36	1, $\frac{1}{32}$ c.c.	73			
37	$\frac{1}{8}$ c.c.	74	—		

Examination of Epidermis of Malta Fever Patients for M. melitensis.

Method.—Patients were selected with temperatures of 100° F. and upwards in different stages of the fever from the 15th to 60th day, epidermis from the arms and flanks scraped away with a sharp sterilised scalpel till the dermis threatened pin-point hæmorrhages, the scrapings put in sterilised capsules, taken to the laboratory and there ground up in a small quantity of sterile normal salt solution — (1 c.c.). From this three successive agar Petris were inoculated with one loopful, to the remainder, 5 c.c. of salt solution was added, and the surface of three other agar Petris inoculated by spreading $\frac{1}{4}$ c.c. of this diluted skin emulsion over each, and the whole incubated at 37° C. for 5 days.

Up to the present this method has been applied to twelve cases.

Discrete colonies of the different micro-organisms usually met with in the skin were obtained in every case, but in none of these plates were colonies of *M. melitensis* ever obtained.

Examination of Sweat from Malta Fever Patients for M. melitensis.

1st Method.—A skin surface of forearm washed with spirit soap, then ether, a carbolic pad 1 in 40 kept on 12 hours, then a circle of sterilised (dry 160° C. air) lint placed on this surface, and a sterilised watch glass strapped over it with adhesive plaster. After critical sweating, circle of lint removed, placed between two sterilised watch glasses held in a metal frame, and sent to me at laboratory. There each circle of lint placed in a separate broth tube numbered, dated, and incubated at 37° C. After 5 days' incubation, agar slopes, inoculated zig-zag from each, incubated at 37° C. and examined daily for growth; if sterile, original broth tubes were inoculated with *M. melitensis* returned to incubator for 4 days and then fresh slopes inoculated from them; on these *M. melitensis* invariably appeared, thus proving that sufficient disinfectant to prevent growth of *M. melitensis* had not been carried into circles of lint from disinfection of skin surface. Nineteen sweat swabs from different patients were thus examined. In some cases the tubes remained sterile, in others the agar slopes yielded growth in discrete colonies.

Result.—No *M. melitensis* was ever recovered by this method.

2nd Method.—The critical sweat was collected in sterile pipettes from four different patients, zig-zagged on agar and incubated. The collection was done by the sisters in the ward who were supplied with the pipettes ready for use, and instructed how to break off the points and apply them. They stated it was rare for sweat to collect in such large drops as to admit of collection in this manner, hence specimens were obtained from only four patients.

Result.—No *M. melitensis* was obtained.

3rd Method.—(A modification of the 1st.)—Circles of lint were obtained saturated with critical sweat from Malta Fever patients as in 1st Method, but instead of being incubated in broth tubes were placed each in a 5 c.c. sterile normal salt solution tubes, in which they were thoroughly agitated and ground up with a sterile glass rod, and the resulting fluid plated out in agar Petri dishes both by spreading $\frac{1}{2}$ c.c. of it over whole surface, and by describing a centripetal spiral with a loop full of the fluid. Discrete colonies were always thus obtained after incubation at 37° C.

The critical sweats of seven patients have been thus examined without *M. melitensis* having been obtained.

To see if M. melitensis would Pass any Filter.

It was felt it would be of the greatest assistance in isolating *M. melitensis* if advantage could be taken of its small size to separate it from other larger organisms by means of filtration, and I, therefore, experimented with the following filters as described :—

New filters were used for the first time in each case. Obviously the first indication was to find a filter that would pass *M. melitensis* and later to see if *M. melitensis* would come through it from a mixture of microbes. Bougies were all first tested for imperfections by placing in water and applying air under pressure. *Chamberland F.* was first tried after being sterilised in the autoclave at 155° C. 1 hour. All junctions were luted with paraffin.

July 8. Placed broth emulsion of verified living *M. melitensis* from one agar slope in container, filled up with peptone broth, tightened pinch cock, placed apparatus in incubator at 37° C.

July 9. Broth in flask remains clear, loosened pinch cock and ran in 6 drops from bougie. This was repeated daily till July 30, apparatus being kept in incubator at 37° C. all the time.

July 30. Three agar slopes inoculated with some of filtrate, drawn off with a sterile pipette from flask through side tube and incubated.

August 4. No growth in any of agar slopes. Experiment concluded.

Result.—No *M. melitensis* has either been washed through or has grown through *Chamberland F.*

2nd Filtration Experiment with M. melitensis.

July 7. Took *Chamberland F.* bougie, tested for imperfections in water with air under pressure, cut off porcelain end, heated resulting cylinder to redness in moufle; fitted up to act as filter, first sterilising all glass parts at 180° C. for 30 minutes, then sterilised apparatus in autoclave 30 minutes at 120° C., and finally luted junctions with paraffin.

July 8. Placed emulsion of living tested *M. melitensis* (emulsion in broth from growth on one agar slope) in cavity of bougie and filled up with peptone broth, removing glass rod in rubber cork to allow of escape of contained air; replaced plug of wool in end of tube; replaced glass rod; and placed apparatus in incubator at 37° C.

July 9. Broth coming through filter into cavity of test-tube, displaced air escaping by tube B which had been also plugged with cotton wool.

July 27. Apparatus has now been in incubator 18 days. Inoculated three agar slopes with filtrate obtained by means of a sterile pipette passed down tube B, and placed these in incubator at 37° C.

July 31. Agar slopes have now been in incubator 4 days and remain without growth.

Result.—*M. melitensis* does not pass Chamberland F.

3rd Filtration Experiment with *M. melitensis*.

To see whether *M. melitensis* will pass any of three Berkefeld filters N., V., and W., of differing porosities (these were obtained from the Lister Institute).

One of each porosity was taken, tested in water with compressed air, sterilised, and fitted up, glass container being first sterilised by boiling in water and then in hot air 1 hour at 160° C. An air pass being arranged in rubber collar to allow of air displaced by filtrate escaping from container. Then the whole sterilised in autoclave at 115° C. for $\frac{1}{2}$ hour.

August 7. Eight cubic centimetres of 5 days' old verified broth growth of *M. melitensis* placed in each bougie with a sterile pipette.

August 8. Some filtrate in container, 8 c.c. more of same broth culture placed in each bougie.

August 9. Five cubic centimetres more of same culture in each bougie.

August 10. Five cubic centimetres more of same culture in each bougie.

August 11. Now placed in incubator at 37° C.

August 22. Inoculated two glucose-litmus-agar slopes from contents of each container. Placed in incubator at 37° C.

September 3. No growth in any of slopes of 22nd. Experiment concluded.

Result.—*M. melitensis* will not pass any of Berkefeld filters N., V., or W.

4th Filtration Experiment with *M. melitensis*.

To see if *M. melitensis* will grow through Berkefeld filters N., V., or W.

One of each porosity taken and treated as in 3rd filtration experiment, and sterilised in autoclave.

August 14. Placed in each bougie with a sterile pipette 5 c.c. of a verified 4 days' broth culture of *M. melitensis*.

August 15. Five more cubic centimetres of same *M. melitensis* broth culture placed in each bougie.

August 16. Filters now working well; V. cylinder being one-third full of filtrate with its bougie immersed in same for $\frac{1}{2}$ inch, W. and N. bougies are only just touching surface of filtrate, so 5 c.c. more of *M. melitensis* broth culture placed in each bougie W. and N.

August 17. N. receiver now half full of filtrate, bougie being

immersed for $\frac{1}{2}$ inch. More *M. melitensis* broth culture added to W. bougie only.

August 18. W. bougie now well immersed in filtrate. Placed all three in incubator at 37° C.

August 23. Filtrate in N. and W. decreasing in bulk by evaporation through wool plug. Placed more *M. melitensis* broth culture inside these two bougies. Returned to incubator at 37° C.

August 29. Broth filtrates from B., V., and W. have now been incubating at 37° C. for 11 days, bougies being immersed, and remain free from turbidity. Inoculated two agar slopes from each and placed in incubator at 37° C.

September 3. No growth in any of slopes of 29th. Experiment concluded.

Result.—*M. melitensis* will not grow through any of Berkefeld filters N., V., or W.

To Produce a Pure Agglutinating Serum for Testing M. melitensis (or Growths Suspected to be M. melitensis) by Inoculating Rabbits with M. melitensis.

At first, serum brought by Major Horrocks from Gibraltar, and obtained from a rabbit so inoculated, by him was used for testing all new growths thought to be *M. melitensis*. Later serum obtained from an inoculated monkey, and from the second rabbit in the following three experiments was used :—

1st Rabbit.

June 18. A healthy-looking rabbit was taken, of weight 1310 grammes, and its blood examined for agglutinating action on *M. melitensis*. None was found, and it was injected subcutaneously with $\frac{1}{2}$ c.c. of a 24 hours' growth of *M. melitensis* in broth at 37° C. (verified).

June 25. Agglutination $\frac{1}{10}$ under $\frac{2}{3}$ in obj.

June 28. " $\frac{1}{10}$ " and it was injected under skin of back with a 4 days' growth of *M. melitensis* on one agar slope (verified) emulsified in broth.

July 3. Rabbit found dead. *Post-mortem*. There was slight congestion of intestines, spleen, and peritoneal vessels; liver somewhat patchy, heart normal. Stomach full of green food. No *post-mortem* cultures were attempted as animal had apparently been dead 12 to 16 hours.

2nd Rabbit.

July 4. Verified 2 days' culture of *M. melitensis* on one agar slope at 37° C., made into an emulsion with $2\frac{1}{2}$ c.c. broth, 1 c.c. of this

injected under skin of back of a fawn and white rabbit weighing 1460 grammes.

July 13. Serum agglutinates in a dilution of $\frac{1}{10}$ *M. melitensis* faintly (microscope $\frac{1}{2}$ obj.); all growth on one agar slope (3 days) of *M. melitensis* (from spleen of man) emulsified in broth and injected subcutaneously.

July 21. Serum in a dilution of $\frac{1}{320}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

July 24. Serum in a dilution of $\frac{1}{100}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

July 27. Serum in a dilution of $\frac{1}{100}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

Injected growth from two-agar slope of *M. melitensis* (spleen of man); July 27.

July 31. Serum in a dilution of $\frac{1}{1000}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

August 4. Serum in a dilution of $\frac{1}{1000}$ agglutinates *M. melitensis* visibly to naked eye. Blood had been drawn as required from July 22 onwards.

August 8. Agglutinates *M. melitensis* $\frac{1}{1000}$ visible to naked eye; rabbit now bled to death under ether from carotid by cannula into sterile test-tubes. After separation of serum latter diluted to $\frac{1}{10}$ with sterile salt solution containing $\frac{1}{2}$ per cent. carbolic acid put up in sterile sealed glass capsules and preserved.

Post-mortem.—All organs appear healthy, spleen enlarged. Inoculated to agar slopes each from spleen, liver, kidney, heart's blood and urine.

August 11. Growth on tubes inoculated from *spleen* and *kidneys*, verified as *M. melitensis*. No growth on slopes from liver, heart's blood, and urine.

August 13. Still no growth on slopes from liver, heart's blood, and urine. Experiment concluded.

3rd Rabbit.

July 4. Verified 2 days' culture of *M. melitensis* on one agar slope made into emulsion with $2\frac{1}{2}$ c.c. broth, and 1 c.c. of this injected under skin of black and white rabbit, 11 A.M., July 4.

July 9. Serum does not agglutinate *M. melitensis*.

July 13. Serum in a dilution of $\frac{1}{10}$ agglutinates *M. melitensis* ($\frac{1}{2}$ obj. microscope). One agar tube *M. melitensis* from spleen of man emulsified and injected.

July 15. Rabbit died at 4 P.M. A *post-mortem* was made and liver found enlarged and studded with cheesy tubercles the size of peas. Other organs apparently healthy. Two agar slopes inoculated from each. Heart's blood, liver, kidney, and spleen; 2 c.c. of urine taken

from bladder with sterile pipette and put in 19 c.c. broth. All incubated at 37° C.

July 18. No growth on any of slopes; incubated agar slopes from urine broth.

July 19. Growth on slope from urine broth; found to be a short thick bacillus.

July 21. Heart's blood, kidney, liver, and spleen slopes have now been incubated 6 days. No growth on any of them. Experiment concluded.

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REPORTS
OF THE
COMMISSION
APPOINTED BY
THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,
FOR THE INVESTIGATION OF
MEDITERRANEAN FEVER,
UNDER THE SUPERVISION OF AN
ADVISORY COMMITTEE
OF
THE ROYAL SOCIETY.

PART II.

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APRIL, 1905.

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REPORT UPON THE GENERAL SANITARY CIRCUMSTANCES OF THE MALTESE ISLANDS, WITH SPECIAL REFERENCE TO THE PREVALENCE OF MEDITERRANEAN FEVER THEREIN.

PART I.—GENERAL SANITARY SURVEY.

PART II.—MEDITERRANEAN FEVER.

PART III.—OUTBREAK OF MEDITERRANEAN FEVER IN THE ESSEX REGIMENT.

PART IV.—GENERAL SUMMARY AND CONCLUSION.

By DR. RALPH W. JOHNSTONE, Medical Inspector Local Government Board.

Brief Description of the Maltese Islands.

These islands include Malta, Gozo, Comino, and Cominetto. The two latter are islets, and, save for the hospital for exotic disease on Comino, with its caretaker, are uninhabited.

The island of Malta is about 17 miles long by 8 wide, having an area of 91 square miles. At the census of 1901 the actually resident population was returned as 176,127. This includes a garrison of about 11,000 men. There were besides about the same number of men on the fleet who were not included.

Gozo is about 9 miles by $4\frac{1}{2}$, with an area of 26 square miles. Its population was 20,002 in 1901.

Both islands are covered by low hills, which, with their intervening valleys, leave little or no flat surface. In Gozo the hills are more abrupt, but in neither island is the highest point more than 760 feet above sea level. Every available inch of land is cultivated, but the fields are shut in by high stone walls, which gives the country its characteristic stony and sterile appearance. There are no rivers.

Geologically the formation is an Upper Coralline limestone, under which are successive beds of greensand and marl, overlying a Globigerina limestone (often locally called calcareous sandstone), which again overlies a Lower Coralline limestone. Practically, all the inhabited part of Malta is denuded down to the Globigerina limestone, with occasional outcroppings of the Lower Coralline formation, and one or two patches of alluvium. The north-western and more elevated part of Malta is, however, covered by the Upper Coralline limestone, as is also the higher ground in Gozo.

PART I.—GENERAL SANITARY SURVEY OF THE MALTESE ISLANDS.

Density of Habitation.—The population enumerated on land at the census of 1901 showed an average density of 1671 persons to the square mile, or in Malta 1926 to the square mile, and in Gozo 775. The population was further classified into (a) persons living within the fortified towns—urban area, 55,298 persons to the square mile; (b) persons living close to the fortified towns—suburban area, 3976 persons to the square mile; and (c) persons living in the districts away from the fortified towns—rural area, 749 persons to the square mile. In Senglea, a part of the urban area, the density was as high as 183,932 persons per square mile, and in Sliema, part of the suburban area, 153,297. In addition, the greater part of the rural population in Malta is housed in villages where the density of population is often higher than in many English cities—Mellieha, for instance, has a density of 147,312 per square mile. Overcrowding of houses upon area is supplemented by overcrowding of persons in houses. Taking as a standard of overcrowding more than two persons living in one room in a tenement consisting of less than five rooms, there are 27 per cent. of the total number of persons in the Maltese islands who are living in overcrowded dwellings. (The percentage in England and Wales at the census of 1901 was 12·2.) This overcrowding is largely contributed to by the number of Kerreyas, or common lodging-houses which exist, especially in the fortified towns. In Valetta, with a total population of about 24,000 persons, more than 5000 persons live in Kerreyas. The population is rapidly increasing. In the decade 1892 to 1901 the Maltese-born population increased 11·6 per cent., other British subjects 12·5 per cent., and foreigners 37·1 per cent.; while each year the population around Valetta, and its harbours, especially tends to increase in density.

Dwellings.—All the dwellings are constructed of stone, generally in two storeys with a flat roof, which is utilised to collect rain-water. In the country districts window space is usually inadequate, sometimes, indeed, altogether absent. Owing to the porous nature of the local stone, the older dwellings, where the walls are not properly protected by copings and damp-proof courses, are said to be damp in winter. Most houses have a small yard or garden, in which is found the mouth of the underground tank used for storing rain-water from the roof. The pavement of the yards is nearly always porous, and is often cracked or defective near the tank mouth, where it is most used. The yard surfaces are very often strewn with refuse and the droppings of birds and animals, or soiled with slop water; they are usually drained to the street gutter. The flooring of rooms is generally constructed of porous stone, which forms a fine dust with surface wear. Rabbits, hens, cats, and dogs are kept in the houses, and sheep and cattle are housed in out-buildings, which usually abut on the dwelling-house.

Roads.—The roads are repaired by the Government, except a few which are repaired by the military authorities. Main roads are kept in good order, but owing to the friability of the local stone used as metalting, there is much dust. Bye-roads are often almost impracticable for wheeled vehicles. In the fortified towns scavenging is done by men in the employment of the Government, and surface water drains are provided. Outside the fortified towns street scavenging is neglected.

Excrement Disposal.—The method most generally employed is what is known as the hand-flushed water-closet. This closet is usually a long hopper basin with a syphon trap below, in connection with a cesspit or a sewer. The closet is, as a rule, used for emptying excrement and slops into. It is seldom used in any other way. It is placed in the most unexpected positions, sometimes in the open yard in a niche in the wall, frequently in the kitchen a foot or two from the cooking apparatus, or it may be in a small cupboard (1' x 2' usually) in the external wall of the house, or in the steps leading to the entrance hall from the front door, and sometimes even in the open street. It is very exceptionally found in a special room or in a position where it is likely to be used in the usual manner. In those poorer class houses which have got a special room for the water closet, the room serves often also as a larder or food store. The hand-flushing of these closets is conspicuous mainly by its absence. Water has always been a precious commodity in Malta, and is used sparingly; in addition cesspits are emptied at the owner's expense, at the high rate of 1s. 6d. per 100 gallons. The consequence is that one rarely sees a water-closet basin even moderately clean. They are usually caked with filth, and the surroundings fouled with faecal matter. Again, when a poor proprietor owns a field or garden, he often ceases to make use of the closet, in order to avoid the cost of emptying, or because he values the excrement as manure. As result, the water in the trap evaporates, and cesspit or sewer air gains access to his premises.

The next most frequent method is what may be called the misbla system. A misbla is a dung heap, and it may be placed in the garden or yard, or more frequently in an outhouse adjoining the living rooms, or in an ordinary room in the house. Here all the excrement of the family is carefully preserved for removal to the fields. The abominable stench that may be caused in a dwelling by this system is not easily described. Occasionally a cellar is used as a misbla, and privy seats are placed in the room above. The vessels which are used to convey excrement from the bedrooms to its destination are often left in the house unemptied for several days. On one occasion on asking to see the vessel used for conveying excrement to the garden, I was shown the bucket in which the vegetables for the family dinner were being washed.

There are a few privy middens, and a few modern properly flushed water-closets. The garrison are provided with trough latrines, flushed once or twice a day, but owing to the feebleness of the flush and the defective pattern of latrine used, were often found overfull and offensive. In places where the latrines were flushed by a rush of water through the trough, a filthy and offensive residue was left behind after the flushing, and in places where the basins were flushed from above into the trough, the feeble trickle of water provided was entirely inadequate. The wooden seats of the military latrines are constructed a few inches above the iron surrounding the opening to the basin, with the result that urine is liable to soil the flat iron surface and dry there, after which the dried residue may be blown about by currents of air.

The naval latrines, or "heads," on board ship are of a better pattern, better flushed, and lack the iron cover underneath the wooden seat. The officers' water-closets, which are used also as urinals, are, from their height above the floor, peculiarly liable to contamination of the floors with urine.

Little effort is made to prevent the pollution of open spaces and walls by excrement and urine. The spaces around the landward fortifications of Floriana are especially liable to fouling, on account of the habits of the country people who pass every day into Valetta. Public urinals sometimes have no water supply, and thus become very offensive.

House Refuse Disposal.—There is no regular system for collection of house refuse in Malta. Certain men with carts call at the houses in the morning and take away house refuse for use as manure, but they are in no way bound to take it, and instances arise where they decline to remove refuse containing material not likely to be useful, such as tin cans and broken bottles. Ash-pits or bins are almost unknown. Refuse is thrown into the garden or into the street. Dead animals, vegetable refuse, and other filth may often be seen in the streets, and many complaints have been made by the better class inhabitants on this score. The police, however, seem powerless to enforce their regulations in the matter.

Owing to the lack of proper flushing the house drains and soil pipes become caked with filth, and complaints of smell arising from the inlet ventilators of house drains are frequent.

Sewers.—In some of the towns, for in Malta the villages are really small towns, quite compact and lacking in open spaces, there are sewers which have existed for centuries. They are built of, or cut in the porous rock, being more in the nature of galleries than sewers, and they are not in any way rendered impervious. They act as elongated cesspools where the liquid sewage gradually soaks away into the rock, leaving a semi-solid residue. Such, for instance, is the system at Birchirchara, Victoria, and formerly at Curmi.

Modern sewers exist at Valetta, Cospicua, Senglea, Vittoriosa, Calcara, Misida, Curmi, and Sliema, and are in course of construction at Notabile and Rabato, and at Hamrun. Many of the houses abutting on the harbour still drain into it, so that when the fleet is in, and many ships are discharging their sewage into the Grand Harbour, the water is liable to become visibly polluted with excremental matter. Since there is very little tide, this may become a serious nuisance.

Cesspits, in the case of new houses, are built under the street and are ventilated and cemented. In the old houses the cesspits are often under the dwelling rooms and unventilated. Very frequently they are placed close to the water tanks. Many old cesspits are never emptied, since they are not impervious, and it is to be feared that new cesspits are occasionally tampered with so as to render them pervious, after they have been passed by the sanitary authority. Cesspits are emptied by means of a pumping engine into iron tank carts. The contractor who undertakes this work is empowered to charge at the rate of 1s. 6d. per 100 gallons.

Water Supply.—There is a public water supply laid on to every village in Malta except Mellieha. The water is derived from groups of springs in three different localities, which afford an approximate mean daily yield of 418,500 gallons by gravity. In addition, there are three other sources from which potable water is pumped, the mean daily yield being about 693,000 gallons. Besides the drinking water there is a brackish water found at Armier, and pumped to Valetta, where it is used for watering streets and flushing sewers. The main storage reservoirs for drinking water are at Ta Kali, near Attard, and are capable of holding 16,865,200 gallons. The total storage capacity of the island is 18,980,000 gallons. The gathering grounds for the water supply are for the main part in thinly populated portions of the island. The water is collected in galleries driven in the rock deep below the surface, and conveyed by iron pipes to the tank or pumping station. From there it is distributed by cast-iron pipes or by stone channels built in, and lined with cement. The water is usually distributed by means of stand-pipes in the villages. In Valetta the houses generally have taps, but they are often without them, and outside the area surrounding the harbours taps are seldom found in the houses. In some villages there are large underground tanks provided by the roadside, which are filled in the winter from the public water supply. These tanks are seldom fitted with pumps, and in consequence become very foul from the constant lowering into them of buckets which have been allowed to stand on the roadside. The vicinity of pervious cesspools provides a possibility of pollution which is often present.

Practically every house in Malta has underneath it a large tank for collecting rain-water from the roof. This water is generally preferred to the public water supply for drinking, possibly because it is cooler

in summer. The contents of these tanks very often show signs of pollution, nor can this be wondered at when it is considered that the roof is very much used by the family in summer, and by their cats and dogs. Tanks are never supplied with pumps, and the bucket which is lowered into them by a rope is often placed on the yard flags amidst slop water and pollutions due to animals or to the neighbouring water-closet. There are very few wells in Malta.

In Gozo the public water supply is obtained from similar sources to that of Malta, the total mean daily supply amounting to about 143,000 gallons, derived from three principal sources. About 90,000 gallons of this water, coming from two sources, is brackish, and about 50,000 gallons, coming from the other source, is good water. The two qualities are mixed before distribution, and the result is a water containing considerable quantities of magnesium salts, which is liable to affect new comers prejudicially.

In the villages of Xahra and Nadur there are wells, a few also being found in Victoria; but elsewhere the usual rain-water tank system prevails, and is liable to the same dangers as in Malta.

All the villages in Gozo except Xahra, Zebbug, Nadur, and Kala have the public water supply, and Nadur is about to be supplied.

Hospitals.—There is a hospital for infectious diseases in connection with the Lazaretto on Manoel Island. It is intended for the isolation of small-pox, scarlet fever, diphtheria, and erysipelas. The buildings are out of date.

The Central General Hospital is at Floriana. It has 226 beds, including the Seamen's Hospital, which adjoins it. It receives cases of enteric and Mediterranean Fever, besides surgical and other cases. No attempt is made to isolate the Mediterranean Fever cases from the others. The methods of this hospital in the matter of cleansing the patient and disposal of the contents of bed-pans of enteric and Mediterranean patients are unsatisfactory. Reference will be made to this matter later. There is no proper hospital sink, and the laundry is inadequate. All infected clothing is despatched to the poor-house laundry. It is said to be steeped in corrosive sublimate solution before being sent, but I saw no signs of the process at my visit, except some barrels containing water, which were shown me as the receptacles used for steeping. There is a general hospital at Citta Vecchia, known as the Santo Spirito Hospital, containing some 70 beds, and there is a similar institution in Victoria, Gozo, with about 60 beds.

The quarantine hospital on the island of Comino is well isolated. It is intended only for ship-borne cholera, yellow fever, or plague.

There is a large building at Marfa, well isolated, which is intended to serve for cases of exotic disease amongst the inhabitants of Malta.

The military hospitals are seven, six in Malta and one in Gozo. The Station Hospital, Valetta, accommodating about 200 patients, is the

ancient hospital of the Knights of St. John. The building is unsuitable for a modern hospital. It contains no hospital sink. Attached to it is the hospital of the Royal Malta Artillery.

The Royal Naval Hospital at Bighi contains about 200 beds.

Sanitary Administration.—There is a Council of Health, consisting of twenty members, six of whom are medical men. It is their duty to advise the Government on sanitary regulations, on quarantine measures, on public works in connection with hygiene, including drainage and water supply, and on all other matters of public health. The Council has power to suggest measures, inquiries, and scientific investigations in connection with public health. The Council meets every two months.

The public health department is directed by the Superintendent of Public Health, Mr. R. P. Samut, M.R.C.S. Eng., who receives £400 per annum. It is his duty to watch over and direct the medical officers of health, the sanitary inspectors, and all other officers of the department, and to advise them. He has also to inspect at intervals the hospitals, quarantine establishments, slaughter-houses, charitable institutions, prisons, etc. Finally, he has to advise the Governor, when required, on public health questions, and he has to draw up an annual report on the sanitary state of the islands, and send it to the Governor.

The Superintendent of Public Health thus reports direct to the Governor, and the functions of the Council of Health are purely advisory, or at most suggestive. The Superintendent solely is responsible for the annual report. His position is one of great responsibility, demanding experience and a high degree of expert knowledge.

There are two medical officers of health for Malta—Dr. Caruana Xicluna, who receives £350 a year, and Dr. F. Xuereb, who receives £250. These gentlemen divide their duties—the first-named superintending buildings, zymotic diseases, foods, shops, and noxious trades, while the latter looks after drainage, notifications, isolation, disinfection, and overcrowding.

There is a medical officer of health for Gozo—Dr. E. Calleja, who receives £120 a year. The Sanitary Engineer, Mr. C. Mallia, receives £170, with £30 additional as superintendent of drains. There are 18 sanitary inspectors in Malta, receiving about £60 a year each. In Gozo there are four, who receive salaries on the same scale. There is an inspector of markets, who receives £110 a year; Mr. MacFarlane, M.R.C.V.S., who superintends the slaughter-house, receiving £30 a year.

There are 22 district medical officers in Malta, and four in Gozo. Up to 1885 these officers received £10 a year for certain public health duties; but since then each of them receives a lump sum varying from £60 a year to £140 a year to cover all their duties, which are in the main the same as those of our poor-law district medical officers. They

also perform vaccination twice a year free of cost. Their public health duties are to inspect infected premises, and determine whether the patient must be removed to hospital or not; to inspect bad food if called in by a sanitary inspector, and to inspect midwives once a month, and see that their equipment is adequate and clean.

The district medical officers are under the control of the Comptroller of Charitable Institutions, as are also the hospitals and other charitable institutions.

There is a public analyst and bacteriologist, with two assistants. Dr. T. Zammit has held the post since January, 1891.

The annual report of the public health department has of late years been very disappointing. Formerly it contained comments and suggestions, but it is now merely statistical and conveys little information as to the conditions which exist and have to be dealt with by the department.

Some of the sanitary inspectors are hard-working, intelligent men, who know their districts; but not a few are entirely ignorant of the elements of sanitation, and in some cases even of the conditions prevailing in their districts. Close supervision and drastic weeding out is required amongst them. The Government have made a new departure this year in sending three young men to England to be trained as sanitary inspectors. They are to be followed by others if the experiment prove successful. I have great hopes that it will. Some of the sanitary inspectors make house-to-house inspections daily, but others never do so unless a case of infectious disease arise. The poorer people are too ignorant of sanitation to make complaint, so that without frequent inspection grave conditions may be allowed to exist for long periods. The public health department have issued a general order to the sanitary inspectors not to report faults in drainage unless urgent, on the grounds, I was informed, that all the villages would some day be sewered. The order is liberally interpreted by many of the inspectors, and was quoted to me in extenuation of such conditions as cesspits ventilated into living rooms, sewers ventilated into houses by unsealed water-closets, leaky cesspits, etc.

There is a sanitary commission appointed by the Governor for the maintenance and construction of drainage. The Superintendent of Public Works is chairman, and there are six other members, four of whom are medical men.

Notification of Infectious Disease.—There is no payment for notification, and though there is a penalty for neglect to notify, it is difficult to exact, and in point of fact never has been exacted. Many considerations interfere with the accurate notification of Mediterranean Fever. For instance, persons who die of it are not permitted burial in a church, a cherished privilege outside the fortified towns, or a private practitioner wishes to spare his patient the annoyance and

expense of lime-washing and disinfection. In addition, the diagnosis is often difficult, the serum test not generally being applied. The consequence is, only severe cases are notified, and not always these. In the official record of the notifications, the age and sex of the patient is generally unrecorded; in many instances even the name is not recorded, nor the number of the house.

A Maltese medical man of experience told me he did not think more than a third of the cases of Mediterranean Fever that occurred in Malta were notified, and not more than a fifth of those in Gozo. I think this estimate is not far wrong. In addition, I found that many English cases attended by Army doctors were not notified.

The following diseases are notifiable:—Plague, cholera, yellow fever, small-pox, scarlet fever, diphtheria, diphtheritic croup, typhus fever, enteric fever, measles, remittent fever (Mediterranean), febrile puerperal diseases, continued fever (on 7th day), erysipelas, epidemic spinal meningitis, chicken-pox, influenza, whooping cough.

Disinfection.—The usual means adopted are fumigation by burning sulphur, soaking washable materials in corrosive sublimate solution, and lime-washing. Bedding and other articles unsuitable for soaking are not sent to the steam disinfector in cases of Mediterranean Fever, seldom indeed in any disease.

Isolation.—Small-pox and diphtheria are isolated, and the early cases in outbreaks of measles or scarlet fever. The routine adopted in these diseases is as follows:—The case is visited by the District Medical Officer, who reports whether it can be isolated at home or not. If it can be isolated at home, a man is sent to act as health guard, whose business it is to prevent communication between the sick room and the public. If the case has to be removed, a police sergeant is sent with the ambulance.

Mediterranean Fever is not isolated.

Sanitary Law is embodied in ordinances enacted by the Governor with the advice and consent of the Council of Government. They cover much the same ground as our own public health laws, though some of the Maltese ordinances are more stringent. They include regulations as to noxious trades, bake-houses, milk-shops,* buildings, markets, refuse in streets, etc.†

There is a quarantine medical officer, with three assistant medical officers, and a veterinary surgeon.

* The usual source of milk in Malta is the goat. These animals are driven about the streets in flocks, and are milked at the customer's door into his own vessel. The udders, which are abnormally large, often touching the ground, are very liable to be soiled. The proprietors of herds are so many that it is always difficult to ascertain from a householder where he has got his milk. No regulations are in force for the effectual control of these vendors.

† The regulation against throwing refuse and offal into the streets is not enforced, or very feebly so.

PART II.—MEDITERRANEAN FEVER.

Introductory.—I do not propose in this report to deal with the history and literature of Mediterranean Fever, or with its symptoms, treatment, or distribution outside Malta, except very briefly, and in so far as these have a direct bearing upon my own part of the work of the Commission.

The study of Mediterranean Fever, virtually commenced by Marston's paper in 1861, received its great impetus from the discovery of the *Micrococcus melitensis* by Bruce in 1887. After this, for many years the difficulty of diagnosis caused by the strong resemblance between this fever and other diseases endemic in Malta, such as enteric fever and the fever known as "simple continued," retarded investigation and detracted from the value of the figures recorded.

In 1897, Wright and Semple, by introducing the serum agglutination test, placed matters on a more exact basis. This method was in 1900 adopted as a routine practice in the Army, and shortly after in the Navy.

Since the publication of Hughes' book in 1897, Mediterranean Fever has attracted considerable attention in the Army and Navy, and much has been written about it. With the exception of Zammit's paper in 1902, little appears to have been done in the way of studying its behaviour amongst the civil population of Malta, either by the local medical men or by others. It was partly for this reason that I devoted most of my attention, during my stay in Malta, to the disease as it occurred amongst the Maltese.

From the outset I found myself confronted by two difficulties, the first, a badly administered system of notification, which has been already referred to in Part I of this report, and closely allied with it considerable unreliability of diagnosis, due partly to the fact that the serum agglutination test is not generally in use in Malta outside the Army and Navy, in spite of the facilities afforded by the Bacteriological Laboratory at Valetta. My second difficulty was the fact that very few Maltese speak English, and that their natural politeness leads them generally to try and give the reply they deem most likely to please, and not that which is most strictly in accordance with the facts. Add to this a strong distrust of the sanitary authority, whose objects they are in general unable to understand, and whose visits they regard solely as a probable source of expense to themselves in the way of white-washing or cleansing, and it will be understood that accurate information was not always easy to come by.

The Geographical Distribution of Malta Fever will be of importance in the future study of the conditions which favour the spread of the disease.

The following list of places, from which Mediterranean Fever has been reported, is taken, with slight additions, from the "Journal of the R.A.M.C.," vol. ii, No. 4:—*Spain*—Gibraltar; *Islands of the Mediterranean*—Balearic Islands, Corsica, Sardinia, Sicily, Malta, Gozo, Cyprus, Crete; *Italy*—Rome, Naples, Caserta, Benevento, Campobasso, Aricca, Terano, Fermo, Padua, Cittanova, etc.; *Greece*—Athens, Cephalonia; *Turkey*—Constantinople, Smyrna; *Palestine*—Jerusalem; *Africa*—Tunis, Algiers, Alexandria, Suakin, Massowah, Zanzibar, Kimberley (?); Aden; *India*—Calcutta, Mian-Mir, Nowshera, Secunderabad, Simla, Delhi, Lucknow, Agra, Allahabad, Choabattia, Subatha, Assam, Swat Valley; *China*—Hong-Kong, Philippine Islands, Fiji Islands; *North America*—Mississippi Valley; *West Indies*—Cuba, Puerto Rico; *South America*—Venezuela, Brazil, Montevideo.

This list will probably undergo considerable enlargement and alteration in the future, and I will only say in connection with it that there are factors which I am not in a position to take into account, such as the reliability of the diagnosis or of the cultures by means of which the diagnosis has been confirmed, and the fixing of the place where infection took place, having regard to the prolonged liability to relapse.

Hitherto, Mediterranean Fever has not been reported north of the 45th parallel or south of the 40th.

Incubation Period.—The general impression amongst Maltese medical men seems to be that the usual incubation period of Mediterranean Fever is not more than 8 or 10 days.

The following cases have occurred in the course of laboratory work with the *Micrococcus melitensis* in places where there was no prevalence of Mediterranean Fever and no apparent source of infection other than in relation with infective material in the laboratory:—

	Incubation period.
1.—S. From an accidental prick with a syringe needle which contained a living culture	15 days
2.—W. From purposeful hypodermic injection of a living culture	16 "
3.—B.S. From accidentally drawing into the mouth a small quantity of living culture through the mouth.....	8 "
4.—E. From the same kind of accident as 3	6 "
5.—S. From accidental wound of the conjunctiva with a portion of a broken tube which had contained living culture*.....	5 "

Besides the above, Fleet-Surgeon Bassett-Smith has informed me of

* In this case, examination by an oculist, soon after the breakage of the tube, failed to disclose any wound of the conjunctiva.

another laboratory case in which the occasion of inoculation could not be traced.

The five cases given above are too few to afford sufficient basis for trustworthy induction. They are, moreover, open to the objection that infection did not necessarily take place on the occasions cited, but may have occurred at some other time in the course of work with infective material. But after due allowance made for the latter consideration, No. 2 is to be regarded as of materially greater value as a guide to the incubation period of Mediterranean Fever than any of the others, since, in this instance, there was a definite and purposeful introduction into the system of a presumably sufficient amount of living culture of *Micrococcus melitensis*, accompanied by record of the time of such introduction, and by subsequent outlook for the first appearance of illness. The remaining four cases, which are marked by considerable diversity in respect of the incubation periods inferred, are more open to challenge, and, therefore, afford less trustworthy guidance in this matter than does No. 2.

I have made inquiry with a view to finding how many cases of Mediterranean Fever have been observed to occur on board our ships of war after leaving a Mediterranean port and before touching at another Mediterranean port.

Fleet-Surgeon Bassett-Smith has kindly examined his records and sent me 13 cases which occurred on board ship, but none in which it could be confidently said that the fever occurred more than 14 days after leaving the last Mediterranean port of call. He included three cases in which Mediterranean Fever occurred three weeks after the ship left Malta, but he was unable to say she had not subsequently called at a Mediterranean port before the onset of the fever. He also included a case in which Mediterranean Fever occurred 12 months after leaving the Mediterranean, but said that the patient had a slight attack of fever while on the Mediterranean station, so that the possibility of a relapse cannot be excluded.

Fleet-Surgeon Bassett-Smith also sent me the following remarkable case:—A. B. left Gibraltar December 20, 1903, having had no attack of fever at the time and feeling quite well. He arrived home for Christmas, went on leave for a month, and then took on duty attached to the "Excellent" gunnery establishment at Gosport. About February 20, 1904, acute fever set in, for which he was sent to the Royal Naval Hospital, Haslar, as a case of enteric fever. His blood was examined in the first fortnight, and gave a negative reaction for enteric, but a positive for Mediterranean, and later on the *Micrococcus melitensis* was isolated from his blood. This case must have had an incubation period of two months if it be conceded that infection occurred in the Mediterranean. There is the possibility that he was infected from some unrecognised case at Gosport, but this possibility

is largely discounted by the fact that many cases of Mediterranean Fever are annually treated at the Royal Naval Hospital, Haslar, without any recorded spread of infection. The case, though exceedingly interesting, will be of greater value in the consideration of the incubation period of Mediterranean Fever, should other like cases occur hereafter ; at present it stands alone.

From Staff-Surgeon Gilmour I received record of eight cases which occurred on warships at sea, and two others I saw myself in Malta, but none of them occurred more than 14 days after the ship had touched at a Mediterranean port.

Ships while on the Mediterranean station are seldom or never more than a few days without touching at a port, so that their records are unlikely to afford any guidance until they leave the station for home.

I received from Lieutenant-Colonel Rhodes, R.A.M.C., details of a search which he caused to be made, at my request, in the records of the R.A.M.C. at Malta for the period January 1, 1901, to August 31, 1904. During that time only two cases could be found in which Mediterranean Fever had occurred less than 14 days after the patient's arrival in Malta. The first case was a Munster Fusilier, who was admitted to hospital suffering from Mediterranean Fever on February 22, 1901, eight days after the regiment's arrival in Malta, from England. The second case occurred in the Sussex Regiment, and was admitted to hospital on July 7, 1904, 11 days after the regiment's arrival in Malta, from England.

Further evidence is required before a definite average incubation period can be established. It may, however, be provisionally stated that the data available tend, in some degree, to suggest that the incubation of Mediterranean Fever ranges about a period of 14 days.*

Distribution of Mediterranean Fever in the Maltese Islands.

Mediterranean Fever occurs in every part of Malta and Gozo, on the sea coast and inland, though as a rule its relative incidence is less severe in the villages more remote from the capitals.

The figures given below with regard to cases have been abstracted from the civil official notification returns, where Mediterranean Fever is generally notified under the name of remittent fever. There are in these returns numerous cases notified under the name continuous fever. These Zammit has included in his returns as Mediterranean Fever cases. His grounds are that in Malta all fevers lasting more than a week are notifiable by law, and since all the named fevers—

* Bruce says that cases have occurred in as short a time as 6 days after arrival in Malta. Hughes, while stating his belief that the incubation period is sometimes as short as 3 days, considered that 10 to 15 days is the most usual time.

such as enteric, etc., are notified separately under their proper headings, the residue returned as continuous fever are in reality Mediterranean Fever. He does not, however, reckon with the fever known as "simple continued fever," the most common form of illness in Malta during the hot weather, or else he does not consider that simple continued fever commonly lasts more than a week.

I have examined the records of the naval hospital in Malta, where the diagnosis is at least as careful as in Malta generally, and I find that during the five years 1897 to 1901 the average duration of cases returned under the heading "Other Continuous Fevers" is over eight days. Practically, all these cases must have been "simple continued fever," since Mediterranean Fever, enteric, malaria, and other named fevers are placed under separate headings. I think it, then, more probable that the majority of the cases noted in the Maltese notification returns as continuous fever were cases of "simple continued fever" than that they were cases of Mediterranean Fever. I have, therefore, included in tables dealing with cases of Mediterranean Fever only those cases which were entered in the Maltese notification records as remittent fever, or as Mediterranean Fever.

Table I (pp. 18 and 19) shows the number of cases of Mediterranean Fever which occurred in each of the districts of Malta and Gozo amongst the civil population during each year of the period 1894 to 1903, together with the mean estimated population of each district during the same period, the average number of cases of Mediterranean Fever per 10,000 of population per year, and the number of deaths from Mediterranean Fever during the whole period.

The very general distribution of Mediterranean Fever throughout Malta is perhaps the most striking feature of this table. It will be seen, too, that it is by no means the localities closest to the harbours which suffer the most severely. Hamrun, a somewhat squalid suburb, and the combined villages of Lia, Attard, and Balzan show the heaviest incidence, while Valetta and the three fortified towns, Cospicua, Senglea, and Vittoriosa, are amongst the least severely attacked. In some respects, however, the latter four places cannot fairly be compared with the remainder of the island. All four towns are paved, drained, and scavenged, a state of affairs not found in any other part of the island.*

Disregarding Valetta and the three fortified towns above mentioned, it will be found that the severity of incidence in Malta depends roughly on the amount of the population. The average incidence throughout

* Floriana is drained but not paved, and the scavenging is not so well carried out as in Valetta; moreover, a vast number of country people pass through it each morning to reach Valetta, and complaints have been made that they use the fortifications around Floriana as a latrine. Curmi was sewered in 1901, Sliema in 1902, and Misida and St. Julian's in 1903.

Malta as a whole, is 32 cases per year per 10,000 of population, and the average for places other than the four towns first mentioned is 37·8, neglecting the cases which occurred in public institutions. The average number of inhabitants for each of these places is 4240. Twelve of them contain more than 4240 persons each, and in 8 of the 12 the average incidence is greater than 37·8. Fourteen districts have less than 4240 inhabitants, and in 12 of them the average incidence is less than 37·8 per 10,000. The mean incidence on the group with over 4240 population is 41·6 per year per 10,000 during the 10-year period, while that upon the group with less than 4240 population was for the same period 26·7 per year per 10,000. This is no doubt a very rough classification, and there are notable exceptions, such as Curmi. On the other hand, the notification returns dealt with are not sufficiently reliable to justify any but the most general groupings, perhaps not even these. The classification, such as it is, would tend to show that, outside Valetta and the towns named, the greater the aggregation of inhabitants living in one locality, the greater is the proportional number of cases of Mediterranean Fever that occurs amongst them. The returns from Gozo do not show the same result, but then the numbers dealt with are smaller and the returns themselves probably more inaccurate than in Malta.

Density of population upon area outside Valetta, Cospicua, Vittoriosa, and Senglea appears to have some influence on the incidence of Mediterranean Fever. Floriana, Hamrun, Misida and Pietá, Sliema, Zeitun, and Mellieha are the most densely populated, all having more than 100,000 persons to the square mile. With the exception of Mellieha, and in a lesser degree Zeitun, these places all have a case incidence above the average. On the other hand Zebbug, Sigguei, Axiak, Gudia, Chircop, Safi, Zurrigo, and Krendi are the least densely populated, each having less than 30,000 persons per square mile, and the proportional incidence on all these places, except Zebbug, is less than the average.*

For purposes of comparison, it has been customary for some years past to divide Malta into three areas—(1) *an urban drained area*, comprising Valetta, Floriana, Cospicua, Vittoriosa, and Senglea; (2) *a suburban undrained area*, comprising Misida, Pietá, Sliema, St. Julian's, Hamrun, Birchirchara, Curmi, Zabbar, Tarxien, and Paola; and (3) *a rural area*. Comparing the three areas for the period 1894 to 1903, it appears that the average number of cases of Mediterranean Fever per year per 10,000 inhabitants was—

(1) Urban drained area	18·8
(2) Suburban undrained area	41·8
(3) Rural area	33·4

* It will be seen later, when considering the maps, that density of population upon area in parts of districts, does not show the same influence.

Table I.

	Number of cases of Mediterranean Fever notified.										Mean estimated population during the period 1894-1908.	Average number of cases of Mediterranean Fever per 10,000 of mean estimated population during the period 1894-1908.	Number of deaths from Mediterranean Fever during the period 1894-1908.
	1894.	1895.	1896.	1897.	1898.	1899.	1900.	1901.	1902.	1903.			
Malta—													
Valetta	17	16	52	23	25	39	44	49	46	30	23,062	14.3	53
Floriana	16	7	34	28	18	43	31	23	27	38	5,910	45.7	20
Misla and Pietà	5	1	3	20	19	26	21	24	60	29	3,648	57.1	6
Sliema and St. Julian's	44	24	53	57	33	55	43	43	67	53	10,856	43.3	39
Hamrun	4	38	71	86	73	92	81	104	34	24	9,364	64.8	26
Coopitua	6	3	74	27	12	16	9	21	8	25	12,128	16.6	31
Viktoria	1	6	7	13	37	33	28	14	13	14	7,182	22.9	16
Senglea	—	1	30	14	11	7	2	4	8	7	8,012	10.5	10
Notabile and Rabato	14	5	8	17	32	76	14	16	36	25	8,045	80.2	36
Dingli	—	—	—	—	—	4	7	8	16	—	759	39.5	2
Zebbug	3	24	53	17	18	13	68	19	28	47	5,416	53.5	21
Siggewi	2	—	4	10	16	33	8	3	6	4	3,192	26.9	9
Bircchirah	10	28	30	25	37	108	67	58	41	43	8,144	54.2	43
Lia, Attard, and Balzan	38	27	58	19	12	52	25	35	31	38	4,523	73.0	20

Naxaro	6	6	16	4	16	11	21	10	14	109	3,443	31.7	6
Musta	5	13	8	32	27	24	24	27	24	191	4,676	40.9	27
Gavur	—	—	—	—	3	7	1	6	4	31	1,355	23.9	5
Mellieha	—	3	—	5	3	7	5	3	1	26	2,233	11.6	4
Cumri	2	1	—	4	3	5	17	8	6	48	7,994	6.0	9
Luca	—	1	—	2	—	3	2	6	12	26	3,318	7.8	5
Tarxien and Paola ..	6	7	8	5	16	8	8	24	17	101	4,476	22.8	15
Zurricco	6	13	11	13	23	8	4	5	3	87	3,589	24.3	8
Safi	2	2	2	—	2	—	1	2	2	11	350	31.4	1
Krendi	1	7	7	6	5	1	1	1	—	31	1,559	19.9	5
Micabbe	1	10	3	2	2	—	—	2	2	23	1,192	19.3	1
Chircop	—	3	—	3	1	1	1	1	2	14	619	32.6	1
Zeitun	55	31	44	10	8	17	14	7	22	222	7,493	29.8	12
Zabbar	47	29	18	17	18	14	24	13	27	214	6,549	38.6	20
Axiak	9	5	1	1	1	4	6	1	1	33	1,493	22.1	1
Gudia	3	9	3	2	3	4	—	—	4	30	1,141	26.3	1
Public institutions ..	9	15	13	6	27	43	23	14	7	173			1
Total	310	247	630	455	748	608	572	551	518	5184	160,803	32.0	452
Gozo—													
Victoria	4	11	4	9	10	2	6	—	13	71	6,101	11.1	3
Garbo	—	—	—	—	1	3	—	—	—	5	1,708	2.9	5
Zebbug	—	—	—	1	2	—	—	1	1	5	1,166	4.3	—
Sannat	—	—	—	7	7	8	—	—	1	25	1,093	22.9	4
Xahra	3	2	2	28	18	23	18	5	2	146	2,509	58.2	7
Xeuchia	—	—	51	6	2	2	2	7	9	43	1,718	25.0	2
Nadur and Kala	2	1	10	—	3	1	24	14	7	53	4,079	13.0	7
Ghamsielem	—	—	1	1	1	—	3	10	1	18	1,106	16.3	2
Ospizio	—	1	—	—	5	2	—	—	1	9			
Total	9	5	68	52	49	40	53	37	35	375	19,480	19.3	30

Considerably below the average number of cases occur in the urban area, and considerably above the average in the suburban area, while the rural area suffers in much the same degree as Malta taken as a whole.

The urban area is better drained and paved, scavenging is more efficiently carried out, and it has more public conveniences than other parts of the island. On the other hand, there is in the urban area equal or greater aggregation of persons in one locality, and more overcrowding upon area. There is no marked difference between the urban and suburban areas as a whole in the matter of the wealth and station of their inhabitants. Parts of Valetta such as the Manderaggio contain the very poorest of the population, and these parts do not appear to suffer out of proportion to their numbers. The three cities also have many very poor inhabitants; but Sliema and St. Julian's are probably the most wealthy and fashionable parts of the island.

There are some stumbling blocks in the way of all attempts to generalise from the table above. The most striking is the difference in the incidence upon Curmi and upon Birchirchara. These two towns, for they are really small towns rather than villages, are situated close together, on the same kind of soil, with the exception that a part of Curmi is on alluvial soil. They contain about the same number of inhabitants, and the same kind of houses, any difference being that there are more good houses in Birchirchara, yet the incidence of Mediterranean Fever upon the latter has been nine-fold as great as upon Curmi during the period 1894 to 1903. I have examined these villages from many points of view, but I have hitherto found nothing that would account to my mind for the difference. It may be that the personal factor is of unusual weight here in the matter of notification. I hope, however, to have some more facts to consider in this connection when the returns which are now being collected in Malta are due for examination.

I prepared a map for the purpose of studying the distribution of Mediterranean Fever during the period January 1, 1899 to July 31, 1904, in Valetta and Floriana respectively.

In Valetta there is no marked aggregation of cases in proportion to population. The apparent aggregations of cases in the Manderaggio, in Strada S. Giuseppe, and in Str. Pozzi are due simply to the fact that these localities are very thickly populated, consisting mainly of houses each of which contains many families—"Kerreyas" or common lodging-houses. I was informed, for instance, that about 3000 people live in the Manderaggio.

In Floriana, also, most of the groupings of cases are due to cases occurring in different families living in the same "Kerreya." There is, however, a remarkable immunity from attack noticeable in Piazza

Maggiore, Piazza Britannica, and Strada Giardino, all of which face on to broad open spaces, and consist in the main of better class houses. This may be partly explained by the fact that the English doctors do not as a rule comply with the notification law. Most better class streets in Floriana, Sliema, and St. Julian's are occupied to a considerable extent by English people, who are generally attended by English doctors.

Another map showed the distribution of Mediterranean Fever in Hamrun during the period January 1, 1892 to July 31, 1904. Here there are not so many "Kerreyas," and in consequence there is less apparent grouping of cases than in Valetta and Floriana.

A third map showed the distribution of cases during the same period in Sliema. Mediterranean Fever cases here show a distinct preference for the southerly slope towards Sliema Creek as opposed to the northern slope towards the open sea, and the houses in Strada It-Torri, which face the sea, seem to have escaped attack. But many of these houses are occupied by English people, and in any case the streets running down to Sliema Creek are much more densely inhabited.

My maps of Misida and Pietà proved valueless, because the notification returns left so many houses unindicated, the names of the streets only being given.

The other maps in my possession serve only to show the same lack of definite aggregation of cases around a particular centre.

The following table shows the distribution of Mediterranean Fever during the period 1901 to 1903 in the Mediterranean Fleet, including such of His Majesty's ships as have called at Malta in passing through the Mediterranean Sea during that period. (Table II.)

Many of the cases occurred on board ships at considerable intervals of time from the vessel's last call at Malta, and it seemed useful to attempt some discrimination between cases that were possibly infected at Malta and those probably infected in some other port. I have accordingly separated the cases which occurred between 6 days and 20 days after a ship's last visit to Malta, and placed them in a separate column headed "Cases connected with Malta." This admits a certain amount of error, because a ship may have visited other ports and become infected at them during the interval of 20 days. I have no record of the ports visited other than Malta. In a few cases no date was assigned for the onset of the fever, and these I have put in the column referred to, in proportion to the number of days in the year spent at Malta by the ship on which they occurred.

For the three years set out in the table the ships, as a whole, show an incidence of 28.55 per 1000 of strength constantly in Malta, a rate corresponding closely with that of the garrison in Malta during the same three years (28.08 per 1000). The two rates, however, are not really comparable, that of the Navy being calculated on a population

Name of Ship.	1901.					1902.		
	Comple- ment.	No. of days in Malta.	No. of cases of Mediterranean Fever.	No. of cases of Mediterranean Fever connected with Malta.	Men. Days in Malta.	Comple- ment.	No. of days in Malta.	No. of cases of Mediterranean Fever.
H.M.S.—								
Benown.....	758	255	16	14	198,290	758	166	17
Ramillies.....	746	158	34	30	117,868	746	123	7
Cæsar.....	759	211	13	11	160,149	759	190	19
Illustrious.....	759	262	16	15	198,858	759	161	12
Victorious.....	759	133	14	10	100,947	759	170	6
Royal Oak.....	714	204	4	2	147,696	714	60	4
Royal Sovereign....	714	254	19	18	180,356	714	97	8
Empress of India....	714	104	9	8	74,256	—	—	—
Canopus.....	753	219	16	12	164,907	753	209	21
Theseus.....	546	173	10	8	57,158	546	67	12
Andromeda.....	679	209	8	5	141,911	679	142	6
Vindictive.....	429	183	1	0	78,507	429	162	6
Tyne.....	101	266	1	1	26,866	101	231	3
Hibernia.....	676	365	14	14	246,740	729	365	20
Devastation.....	410	156	1	0	63,960	410	5	0
Rupert.....	294	19	13	4	5,586	294	10	0
Diana.....	450	116	7	5	52,200	450	193	16
Hood.....	694	174	7	7	119,756	694	174	21
Harrier.....	122	124	0	0	15,123	124	55	0
Vulcan.....	443	188	5	3	61,184	443	230	8
Pegasus.....	226	95	3	2	21,470	226	117	4
Implacable.....	780	31	3	0	24,180	780	213	25
Gladiator.....	429	138	4	2	59,202	429	216	6
Scout.....	147	72	1	1	10,534	—	—	—
Pioneer.....	224	172	1	0	38,528	224	209	9
Barham.....	175	187	2	1	32,725	175	194	1
Pyramus.....	224	194	9	8	43,456	224	178	12
Surprise.....	107	255	9	9	27,285	107	258	4
Halcyon.....	122	16	0	0	1,952	—	—	—
Dryad.....	121	212	6	5	16,562	121	226	7
Hussar.....	121	154	0	0	18,634	121	176	1
Salamander.....	91	134	0	0	12,194	—	—	—
Speedy.....	91	192	3	3	17,472	91	246	6
Ardent.....	53	217	0	0	11,501	53	169	0
Dragon.....	53	262	0	0	13,886	53	146	0
Kangaroo.....	63	61	0	0	3,843	63	237	0
Desperate.....	63	27	0	0	1,701	63	276	0
Myrmidon.....	63	27	0	0	1,701	63	242	0
Chamois.....	63	56	0	0	3,528	63	276	0
Bruizer.....	53	227	0	0	12,081	53	140	0
Banshee.....	53	227	0	0	12,081	53	290	0
Foam.....	63	260	0	0	16,380	63	233	0
Earnest.....	63	274	0	0	17,262	63	260	0
Griffon.....	63	274	0	0	17,262	63	278	0
Boxer.....	53	246	0	0	13,038	53	97	0
Hardy.....	53	167	0	0	8,851	—	—	—

II.

1902.		1908.					Cases per 1000 complement.		
No. of cases of Mediterranean Fever connected with Malta.	Men. Days in Malta.	Complement.	No. of days in Malta.	No. of cases of Mediterranean Fever.	No. of cases of Mediterranean Fever connected with Malta.	Men. Days in Malta.	1901.	1902.	1903.
14	125,838	758	93	8	5	70,494	21.11	22.43	10.55
7	91,758	746	102	10	9	76,092	45.58	9.38	13.40
15	144,210	759	121	10	8	91,839	17.13	25.03	13.18
7	122,199	759	163	15	13	123,717	21.08	15.94	19.76
4	129,030	770	35	8	2	28,490	18.45	7.91	10.39
2	42,840	—	—	—	—	—	5.60	5.60	—
5	69,258	—	—	—	—	—	26.61	11.20	—
—	—	—	—	—	—	—	12.61	—	—
16	157,877	753	8	5	2	6,024	21.25	27.90	6.64
12	36,682	—	—	—	—	—	18.32	23.96	23.96
4	96,418	—	—	—	—	—	11.78	8.83	—
3	69,496	429	122	12	7	52,388	2.33	13.99	27.97
3	23,381	101	223	2	2	23,028	9.90	29.70	19.80
20	265,085	721	365	21	21	263,065	20.71	27.43	29.96
0	2,050	—	—	—	—	—	2.44	0.00	—
0	2,940	—	—	—	—	—	44.22	0.00	—
11	86,850	450	154	16	13	69,300	15.56	35.56	35.56
20	120,756	—	—	—	—	—	10.09	30.26	—
0	6,820	124	86	0	0	10,664	0.00	0.00	0.00
8	101,890	443	96	4	2	41,528	11.29	18.04	9.08
2	26,442	226	196	3	3	44,296	13.28	17.70	13.28
23	166,140	780	199	23	12	155,220	3.85	32.05	29.49
5	92,664	429	160	5	2	68,640	9.32	13.99	11.66
—	—	149	5	0	0	745	6.80	—	0.00
6	46,816	224	99	3	2	22,176	4.46	40.18	13.39
1	33,950	—	—	—	—	—	11.42	5.71	—
7	39,872	224	181	5	4	40,544	40.18	53.57	22.32
3	27,606	107	202	2	1	21,614	84.11	37.38	18.69
—	—	—	—	—	—	—	0.00	—	—
6	27,346	121	171	16	13	20,691	49.59	57.85	132.23
1	21,296	123	139	0	0	17,097	0.00	8.26	0.00
—	—	—	—	—	—	—	0.00	—	—
6	22,386	91	171	4	2	15,561	32.97	65.93	43.95
0	8,957	—	—	—	—	—	0.00	0.00	—
0	7,738	—	—	—	—	—	0.00	0.00	—
0	14,931	63	248	0	0	15,624	0.00	0.00	0.00
0	17,388	63	272	0	0	17,136	0.00	0.00	0.00
0	15,246	63	191	0	0	12,033	0.00	0.00	0.00
0	17,388	63	61	0	0	3,843	0.00	0.00	0.00
0	7,420	53	16	0	0	848	0.00	0.00	0.00
0	15,370	53	244	0	0	12,932	0.00	0.00	0.00
0	14,679	—	—	—	—	—	0.00	0.00	—
0	16,380	63	164	0	0	10,332	0.00	0.00	0.00
0	17,514	63	208	0	0	13,104	0.00	0.00	0.00
0	5,141	53	80	0	0	4,240	0.00	0.00	0.00
—	—	—	—	—	—	—	0.00	—	—

Table II

Name of Ship.	1901.					1902.		
	Comple- ment.	No. of days in Malta.	No. of cases of Mediterranean Fever.	No. of cases of Mediterranean Fever con- nected with Malta.	Men. Days in Malta.	Comple- ment.	No. of days in Malta.	No. of cases of Mediterranean Fever.
H.M.S.—								
Orwell.....	63	205	0	0	12,915	63	250	0
Coquette.....	63	253	0	0	15,939	63	242	0
Cygnets.....	63	186	0	0	11,718	63	242	0
Conflict.....	53	265	0	0	14,045	—	—	—
Cruiser.....	93	179	0	0	16,647	93	242	3
Imogene.....	42	75	0	0	3,150	53	91	0
Formidable.....	777	28	0	0	21,756	777	90	18
Pandora.....	226	10	0	0	2,260	226	174	0
Irresistible.....	—	—	—	—	—	780	145	5
Repulse.....	—	—	—	—	—	721	117	12
Flying Fish.....	—	—	—	—	—	63	175	0
Goldfinch.....	—	—	—	—	—	94	56	0
Bulwark.....	—	—	—	—	—	829	104	5
Vengeance.....	—	—	—	—	—	762	84	10
Aboukir.....	—	—	—	—	—	755	79	12
London.....	—	—	—	—	—	742	104	3
Hermione.....	318	12	0	0	3,816	324	141	11
Ariel.....	63	28	0	0	1,764	63	242	0
Naiad.....	—	—	—	—	—	276	76	3
*Orion.....	—	—	—	—	—	55	274	6
Panther.....	—	—	—	—	—	63	250	0
Locust.....	—	—	—	—	—	63	250	0
Thrasher.....	—	—	—	—	—	63	299	0
St. George.....	560	5	0	0	2,800	560	13	0
Juno.....	456	5	0	0	2,280	456	13	0
Rainbow.....	—	—	—	—	—	276	13	0
Brilliant.....	—	—	—	—	—	279	13	0
Albatross.....	—	—	—	—	—	69	137	0
Fawn.....	—	—	—	—	—	63	137	0
Mallard.....	—	—	—	—	—	63	123	0
Cynthia.....	—	—	—	—	—	63	126	0
Stag.....	—	—	—	—	—	63	14	0
Bat.....	—	—	—	—	—	63	14	0
Seal.....	—	—	—	—	—	63	14	0
Crane.....	—	—	—	—	—	63	14	0
Venerable.....	—	—	—	—	—	771	10	0
Bacchante.....	—	—	—	—	—	729	11	0
Intrepid.....	—	—	—	—	—	271	2	0
Mohawk.....	178	1	0	0	178	—	—	—
Russell.....	—	—	—	—	—	—	—	—
Montagu.....	—	—	—	—	—	—	—	—
Exmouth.....	—	—	—	—	—	—	—	—
Albemarle.....	—	—	—	—	—	—	—	—

* Since this table was made out, I have been informed that the cases returned as occurring on thus refer to a total complement of 1331 men. This accounts for the apparent absence of cases

—continued.

1902.		1903.					Cases per 1000 complement.		
No. of cases of Mediterranean Fever connected with Malta.	Men. Days in Malta.	Complement.	No. of days in Malta.	No. of cases of Mediterranean Fever.	No. of cases of Mediterranean Fever connected with Malta.	Men. Days in Malta.	1901.	1902.	1903.
0	15,750	63	25	0	0	1,575	0·00	0·00	0·00
0	15,246	63	15	0	0	945	0·00	0·00	0·00
0	15,246	63	191	0	0	12,083	0·00	0·00	0·00
—	—	—	—	—	—	—	0·00	—	—
3	22,506	93	89	1	1	8,277	0·00	32·28	10·75
0	4,823	41	59	0	0	2,419	0·00	0·00	0·00
10	69,990	777	183	18	12	142,191	0·00	28·17	23·17
0	39,224	226	115	1	1	25,990	0·00	0·00	4·45
3	113,100	780	125	14	8	97,500	—	6·41	17·95
6	84,857	721	74	4	1	53,354	—	16·64	5·55
0	11,025	63	263	0	0	16,569	—	0·00	0·00
0	5,264	—	—	—	—	—	—	0·00	—
3	86,216	829	187	10	9	155,023	—	4·02	12·08
3	64,008	762	136	10	10	103,632	—	18·12	18·12
9	59,645	755	131	16	11	98,905	—	15·89	21·19
2	77,168	742	143	29	17	106,106	—	4·04	39·08
9	45,684	324	73	7	4	23,652	0·00	38·92	21·60
0	15,246	63	275	0	0	17,325	0·00	0·00	0·00
2	20,976	271	82	2	2	22,222	—	10·87	7·38
6	15,070	55	365	18	18	20,075	—	109·09	327·27
0	15,750	63	112	0	0	7,056	—	0·00	0·00
0	15,750	63	208	0	0	13,104	—	0·00	0·00
0	18,837	63	208	0	0	13,104	—	0·00	0·00
0	7,280	—	—	—	—	—	0·00	0·00	—
0	5,823	—	—	—	—	—	0·00	0·00	—
0	3,588	—	—	—	—	—	—	0·00	—
0	3,627	—	—	—	—	—	—	0·00	—
0	9,453	69	208	0	0	14,852	—	0·00	0·00
0	8,031	63	221	0	0	13,923	—	0·00	0·00
0	7,749	63	235	0	0	14,805	—	0·00	0·00
0	7,938	63	216	0	0	13,608	—	0·00	0·00
0	882	63	230	0	0	14,490	—	0·00	0·00
0	882	63	235	0	0	14,805	—	0·00	0·00
0	882	63	208	0	0	13,104	—	0·00	0·00
0	882	63	203	0	0	12,789	—	0·00	0·00
0	7,710	771	161	11	10	124,131	—	0·00	14·27
0	8,019	729	164	9	5	119,556	—	0·00	12·34
0	542	271	73	3	1	19,783	—	0·00	11·07
—	—	180	69	3	3	12,420	0·00	—	16·67
—	—	715	107	5	2	76,505	—	—	6·99
—	—	715	59	3	3	42,185	—	—	4·20
—	—	715	24	2	0	17,160	—	—	2·80
—	—	742	14	0	0	10,388	—	—	0·00

board H.M.S. "Orion" include cases which occurred on the torpedo-boat destroyer flotilla, and on board the torpedo-boat destroyer flotilla.

Table II

Name of Ship.	1901.					1902.		
	Comple- ment.	No. of Days in Malta.	No. of cases of Mediterranean Fever.	No. of cases of Mediterranean Fever con- nected with Malta.	Men. Days in Malta.	Comple- ment.	No. of days in Malta.	No. of cases of Mediterranean Fever.
H.M.S.—								
Arethusa	—	—	—	—	—	—	—	—
Thetis	—	—	—	—	—	—	—	—
Hawke	—	—	—	—	—	161	10	0
Spartiate	—	—	—	—	—	—	—	—
Europa	—	—	—	—	—	—	—	—
Sirius	—	—	—	—	—	—	—	—
Victoria and Albert ..	—	—	—	—	—	—	—	—
Minerva	—	—	—	—	—	456	6	0
Venus	452	17	0	0	7,684	—	—	—
Assaye	178	—	—	—	—	—	—	—
Porpoise	178	2	0	0	356	—	—	—
Merlin	—	—	—	—	—	—	—	—
Pique	—	—	—	—	—	—	—	—
Cossack	—	—	—	—	—	—	—	—
Leviathan	—	—	—	—	—	—	—	—
Goliath	—	—	—	—	—	—	—	—
Scylla	—	—	—	—	—	—	—	—
Diadem	—	—	—	—	—	—	—	—
Psyche	—	—	—	—	—	—	—	—
Duncan	—	—	—	—	—	—	—	—
Centurion	606	3	0	0	1,818	—	—	—
Argonaut	—	—	—	—	—	—	—	—
Arrogant	—	—	—	—	—	—	—	—
Furious	—	—	—	—	—	442	6	0
Undaunted	494	1	0	0	494	—	—	—
Ringdove	76	1	0	0	76	—	—	—
Peacock	76	2	0	0	152	—	—	—
Magicienne	224	1	0	0	224	—	—	—
Raccoon	182	1	0	0	182	—	—	—
Linnet	92	4	0	0	368	—	—	—
Pigeon	76	1	0	0	76	—	—	—
Bonaventure	318	1	0	0	318	—	—	—
Marathon	224	1	0	0	224	—	—	—
Dido	450	2	0	0	900	—	—	—
Isis	450	2	0	0	900	—	—	—
Cockatrice	78	1	0	0	78	78	1	0
Melita	125	2	0	0	250	—	—	—
Ocean	751	2	0	0	1,502	—	—	—
Ophir	324	2	0	0	648	—	—	—
Rambler	113	87	0	0	9,831	—	—	—
Blake	128	2	0	0	256	—	—	—
Blenheim	592	2	0	0	1,184	—	—	—
Phoebe	217	2	0	0	434	—	—	—
Perseus	224	1	0	0	224	—	—	—
Talbot	442	1	0	0	442	—	—	—
Lapwing	78	1	0	0	78	—	—	—

—continued.

[illegible]

Name of Ship.	1901.					1902.		
	Comple- ment.	No. of days in Malta.	No. of cases of Mediterranean Fever.	No. of cases of Mediterranean Fever connected with Malta.	Men. Days in Malta.	Comple- ment.	No. of days in Malta.	No. of cases of Mediterranean Fever.
H.M.S.—								
Eclipse	455	3	0	0	1,365	—	—	—
Cressy	754	1	0	0	754	—	—	—
Albion	791	4	0	0	3,164	—	—	—
Fox	324	3	0	0	972	—	—	—
Iphigenia	105	6	0	0	630	105	1	0
Fearless	149	1	0	0	149	—	—	—
Mutine	105	2	0	0	210	—	—	—
Vestal	105	1	0	0	105	—	—	—
Amphitrite	327	3	0	0	981	327	4	0
Rinaldo	—	—	—	—	—	105	2	0
Espiegle	—	—	—	—	—	118	3	0
Daphne	—	—	—	—	—	138	2	0
Brisk	—	—	—	—	—	180	10	0
Aurora	—	—	—	—	—	503	1	0
Redpole	—	—	—	—	—	78	1	0
Plover	—	—	—	—	—	78	3	0
Pigmy	—	—	—	—	—	78	1	0
Astræa	—	—	—	—	—	321	1	0
Orlando	—	—	—	—	—	503	2	0
Endymion	—	—	—	—	—	553	1	0
Terrible	—	—	—	—	—	870	2	0
Majestic	—	—	—	—	—	806	6	0
Magnificent	—	—	—	—	—	799	6	0
Hannibal	—	—	—	—	—	769	6	0
Prince George	—	—	—	—	—	766	6	0
Jupiter	—	—	—	—	—	769	6	0
Mars	—	—	—	—	—	766	6	0
Niobe	—	—	—	—	—	689	6	0
Sutlej	—	—	—	—	—	755	6	0
Doris	—	—	—	—	—	428	6	0
Pactolus	—	—	—	—	—	229	6	0
Prometheus	—	—	—	—	—	229	6	0

Summary of Table II.

Totals.	1901.	1902.	1903.
Number of cases of Mediterranean Fever ..	249	349	338
Number of cases of Mediterranean Fever connected with Malta	198	266	241
Men—days in Malta	2,810,819	3,819,849	2,882,323
Average men constantly in Malta	7,700·87	9,095·48	7,896·77
Cases per 1000 complement	25·71	29·25	30·51
3 years' average	28·55		

-continued.

1902.		1903.					Cases per 1000 complement.		
No. of cases of Mediterranean Fever connected with Malta.	Men. Days in Malta.	Comple-ment.	No. of days in Malta.	No. of cases of Mediterranean Fever.	No. of cases of Mediterranean Fever connected with Malta.	Men. Days in Malta.	1901.	1902.	1903.
—	—	—	—	—	—	—	0·00		
—	—	—	—	—	—	—	0·00		
—	—	—	—	—	—	—	0·00		
—	—	—	—	—	—	—	0·00		
0	105	—	—	—	—	—	0·00	0·00	
—	—	—	—	—	—	—	0·00		
—	—	—	—	—	—	—	0·00		
—	—	—	—	—	—	—	0·00		
0	1,308	—	—	—	—	—	0·00	0·00	
0	210	—	—	—	—	—	—	0·00	
0	339	—	—	—	—	—	—	0·00	
0	276	—	—	—	—	—	—	0·00	
0	1,800	—	—	—	—	—	—	0·00	
0	503	—	—	—	—	—	—	0·00	
0	78	—	—	—	—	—	—	0·00	
0	234	—	—	—	—	—	—	0·00	
0	78	—	—	—	—	—	—	0·00	
0	321	—	—	—	—	—	—	0·00	
0	1,006	—	—	—	—	—	—	0·00	
0	553	—	—	—	—	—	—	0·00	
0	1,740	—	—	—	—	—	—	0·00	
0	4,886	—	—	—	—	—	—	0·00	
0	4,794	—	—	—	—	—	—	0·00	
0	4,614	—	—	—	—	—	—	0·00	
0	4,596	—	—	—	—	—	—	0·00	
0	4,614	—	—	—	—	—	—	0·00	
0	4,596	—	—	—	—	—	—	0·00	
0	4,184	—	—	—	—	—	—	0·00	
0	4,530	—	—	—	—	—	—	0·00	
0	2,556	—	—	—	—	—	—	0·00	
0	1,874	—	—	—	—	—	—	0·00	
0	1,374	—	—	—	—	—	—	0·00	

often absent from Malta during the season when Mediterranean Fever is at its worst.

It appears that when Mediterranean Fever attacks the crew of a ship one year, it is very likely to attack the crew of the same ship in each of the following years that she remains on the station.* There is little relation between the number of days spent by a ship in Malta and the number of cases of Mediterranean Fever which occur

* H.M.S. "Rupert" left the Mediterranean in February, 1902.

on board her, but since no allowance is made in the table for the time of year at which the ship was in Malta, the relation may be closer or less close than is apparent here.

The facts seem to point to the ship herself becoming infected, and in some way assisting in the transmission of infection. Of course, the larger the crew the greater the chance of infection being introduced into the ship from outside. It must be remembered, however, that I am only dealing with a three-year period, which is altogether too short to base reliable conclusions upon.

The data from which the above table is compiled did not reach me until some time after I left Malta, so that I was unable to make investigation by its light on board ships which were at Malta during my visit. It may be said, however, that on larger ships the ventilation is better, and that on Destroyers, in particular, the closets are so constructed that they must be very difficult to cleanse.

The distribution of Mediterranean Fever amongst the garrison during the period 1897 to 1903 is shown in the following table, which

Table III.

	Number of cases of Mediterranean Fever admitted to hospital during the period 1897—1903.	Average number of men in occupation during each year of the period 1897—1903.	Average number of cases admitted per 1000 of strength per year during the period 1897—1903.
St. Francis, Floriana (R.E.) and Ravelin	20	194·86	14·7
Floriana (infantry).....	249	572·14	62·2
Lower St. Elmo (infantry)	134	649·57	29·4
Upper „ (R.G.A.)	46	272·86	24·0
Tigne (R.G.A.)	54	412·57	18·7
Manoel (infantry), including huts ...	138	332·86	22·8
Notre Dame (infantry), including Ravelin and huts.....	49	217·14	32·2
Old laboratory (infantry and A.S.C.)..	17	86·43	28·1
Marsamuscetto (infantry and A.O.C.)	18	74·43	34·5
St. James' Cavalier (R.G.A.)	12	129·86	13·2
Verdala (infantry)	104	534·14	27·8
Imtarfa (infantry and various).....	224	989·29	13·7
Porte de Bombe tents.....	1	No record	available.
Castille (R.A. and R.E.)	1	17·86	8·0
Fort Madelina (R.G.A.)..	1	25·71	5·6
Valetta Hospital (R.A.M.O.).....	29	65·00	65·2
Mellieha Camp.....	33	273·40*	24·1*
Corradino Prison.....	6	16·00	53·6

* For period 1899—1903, not occupied before 1899.

Table III—continued.

	Number of cases of Mediterranean Fever admitted to hospital during the period 1897—1908.	Average number of men in occupation during each year of the period 1897—1908.	Average number of cases admitted per 1000 of strength per year during the period 1897—1908.
Camarata married quarters	15	82·86	25·9
Sliema married quarters.....	7	No record	available.
St. Clements (infantry).....	5	50·00	14·3
Zabbar Gate "	10	98·86	15·2
Zeitun Gate "	4	55·43	10·3
Polverista "	35	156·86	31·9
St. Nicholas "	4	33·71	16·9
Vittoriosa "	3	47·43	9·0
Salvatore " (including C. Guard).....	2	96·43	2·1
Fort St. Angelo (R.M.A. and infantry)	2	33·71	3·4
Couvre Porte (infantry and R.M.A.)	1	51·43	2·8
Inquisitor's Palace (infantry).....	3	7·29	58·8
St. Paul's Bastion (infantry and A.S.C. stables).....	4	26·29	21·7
St. John's Bastion (infantry).....	1	22·43	6·4
Fort Ricasoli (R.G.A.)	40	406·14	11·5
" Rinella "	1	15·86	9·0
" Delimara "	6	23·43	36·6
" San Leonardo (R.A. and R.E.)	3	25·14	17·0
" della Grasia (R.G.A.)	2	13·00	22·0
" San Francesco (infantry).....	2	23·71	12·0
" Isola Gate (infantry).....	8	39·14	29·2
Cottonera Hospital (R.A.M.C.).....	25	44·14	80·9
Pembroke (R.G.A. and infantry)	49	187·14	37·4
Duerra Lines (R.G.A.).....	1	4·57	31·9
Ghajn Tuffieha.....	99	370·87*	89·0*
St. George's Barracks (R.G.A. and infantry)	108	1024·71	15·1
Forrest Hospital (R.A.M.C.).....	1	9·43	15·1
Gozo	24	199·86	17·2
Fort Lascaris (R.M.A.)	29	253·86	16·3
Living in places not mentioned above	0	448·29	00·0
Whole garrison	1625	9037·43	25·6

is compiled from information furnished me by the Director of Transport and Supplies, and by the Principal Medical Officer in Malta. The cases are only those admitted to hospital, and would include all cases occurring amongst the garrison except a few officers who were nursed at their own homes. Of these I could not obtain a record.

The average annual attack rate for the whole garrison, as far as

* For years 1901, 1902, and 1903, not occupied before 1901.

TABLE IV.

Age	1902.					1903.				1902 and 1903.	
	Average strength.	Admissions to hospital on account of Malarian Fever.	Deaths from Malarian Fever.	Ratio per 1000 of strength per year.		Average strength.	Admissions to hospital on account of Malarian Fever.	Deaths from Malarian Fever.	Ratio per 1000 of strength per year.	Ratio per 1000 of strength per year.	Ratio per 1000 of strength per year.
				Admissions.	Deaths.				Admissions.	Deaths.	Admissions.
Under 20 years	1032	14	0	13·6	0·00	1041	67	3	64·4	2·88	89·8
From 20 to 25 years ..	2339	45	1	15·3	0·34	3490	196	1	56·6	0·29	87·7
" 25 30 " ..	1926	18	1	9·3	0·52	1495	53	1	35·4	0·67	20·8
" 30 35 " ..	1498	24	3	16·0	2·00	1117	34	2	80·4	1·79	23·2
" 35 40 " ..	1593	39	0	24·5	0·00	1294	37	1	28·5	0·77	26·3
40 years and upwards.	230	15	1	65·2	4·35	386	17	1	44·0	2·59	57·9
Totals	9218	155	6	16·8	0·65	8738	404	9	45·9	1·02	31·0

this record goes, was 25·6 per 1000 during the seven-year period 1897 to 1903. The heaviest incidence was upon Floriana Infantry Barracks, Valetta Station Hospital, Cottonera Hospital, and Ghain Tuffieha Camp. The two* first places are specially dealt with subsequently; the heavy incidence upon Ghain Tuffieha Camp was occasioned by an outbreak in 1903. A large number of men annually occupy the camp for a few months during the summer only, so that an outbreak occurring amongst them raises the figure representing the annual case incidence out of proportion to the importance of the outbreak itself, because the representative figure is calculated on the average occupation during the whole year.

Age and Sex Incidence could not be ascertained from the civil official returns, but next autumn figures which are now being collected for the year ending July 31, 1905, will be available.

The age incidence for the garrison during 1902 and 1903 is shown as far as possible in Table IV (see page 32).

The two years tabulated show considerable disparity in the number of admissions and in the resulting ratios per 1000 of strength. From the figures of the two years together, it would appear that men under 25 years of age suffer more than the average number of attacks per 1000. From 25 to 40 there is a degree of immunity becoming less with advancing years. Over age 40 the incidence again becomes severe. The deaths are too few to permit of any useful deductions being made from them.

Length of Service in Malta is, as might be expected, closely connected with age in its influence upon the incidence of Mediterranean Fever, as the following table shows (see page 34).

The heaviest incidence of Mediterranean Fever is upon men with less than one year's service; in fact, they are the only class shown with an incidence greater than the average incidence of the disease upon all classes taken together. The incidence upon men with over two years' service is less than half that upon men with under two years' service, and the severity of incidence continues to decrease with length of service up to five years, after which it again rises. In the last three classes shown in the table, however, the numbers dealt with are too small to carry much weight.

The decrease of incidence with length of service in Malta is, no doubt, influenced to a large extent by the elimination of the more susceptible subjects. Further figures will be available next autumn.

The Case Mortality amongst the civil population differs enormously in different localities, as is to be expected when dealing with small numbers and unreliable notification returns. Amongst the civil population of Malta, 8·9 cases per 100 attacked died during the period

* There was an outbreak of Mediterranean Fever in Floriana infantry barracks during 1903. The details were not obtained in time for inclusion in this report.

TABLE V.

Length of service in Malta.	1902.					1903.					1902 and 1903.
	Average strength.	Admissions to hospital on account of Mediterranean Fever.	Deaths from Mediterranean Fever.	Ratio per 1000 of strength per year.		Average strength.	Admissions to hospital on account of Mediterranean Fever.	Deaths from Mediterranean Fever.	Ratio per 1000 of strength per year.		
				Admissions.	Deaths.				Admissions.	Deaths.	
Under 1 year.....	4878	103	3	21.1	0.62	4543	261	2	57.5	0.44	38.6
From 1 to 2 years....	1913	30	1	15.7	0.52	3142	117	5	37.2	1.59	29.1
" 2 3 ".....	1190	10	0	8.4	0.00	550	16	2	28.1	3.64	14.9
" 3 4 ".....	550	6	1	10.9	1.82	259	6	0	23.2	0.00	14.8
" 4 5 ".....	309	3	0	9.7	0.00	173	0	0	00.0	0.00	6.2
" 5 10 ".....	292	2	1	6.8	3.42	106	4	0	37.7	0.00	15.1
10 years and upwards.	86	1	0	11.6	0.00	20	0	0	00.0	0.00	9.4
Totals	9218	155	6	16.8	0.65	8793	404	9	45.9	1.02	31.0

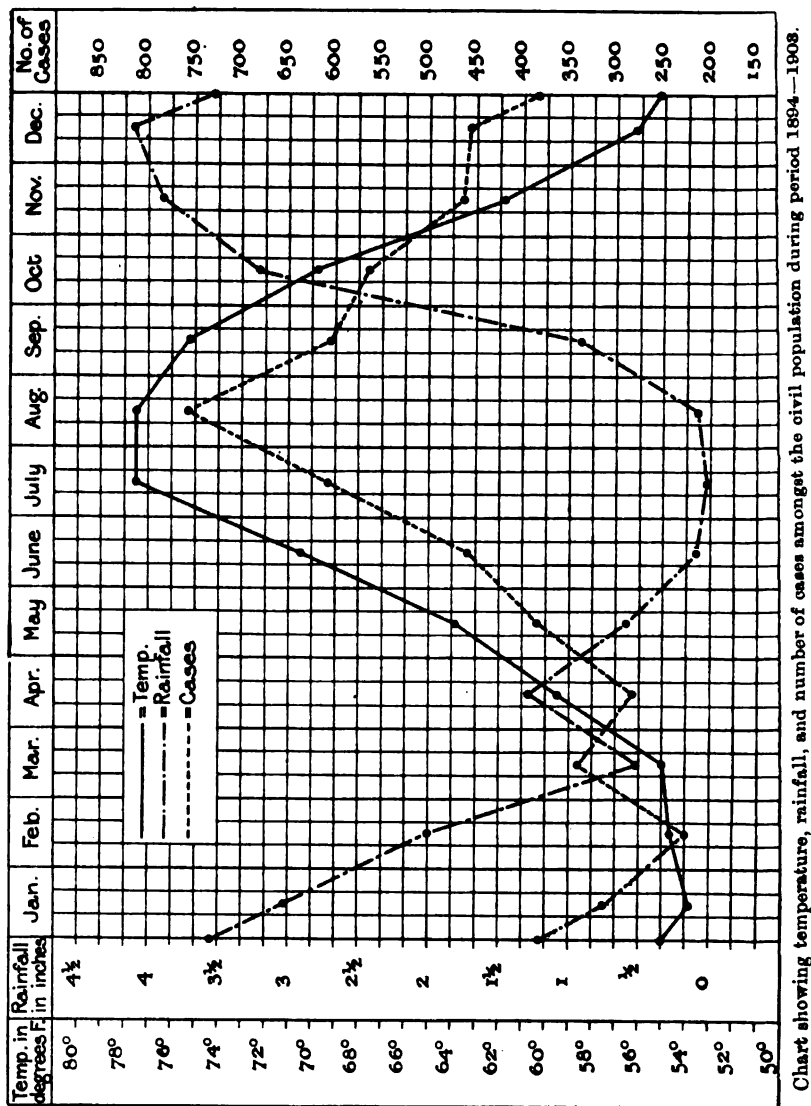
1894 to 1903, and amongst the civil population in Gozo, 8·4 per 100 attacked died during the same period. These figures form a striking contrast to the case mortality of the Army and of the Navy. In the Army, during the period 1897 to 1903, the case mortality was 3·2 per cent., and in the Navy it was only 1·4 per cent. during the period 1897 to 1901. It is probable that the case mortality is higher amongst the civil population than in the Army and Navy, owing to the superior nursing and attention enjoyed by the services, but I do not think it likely that the difference noted above represents the true state of affairs. Probably the high case mortality among the civilians is largely due to the fact that mild cases of Mediterranean Fever more often escape notification than severe ones.

Temperature and Rainfall in Connection with Mediterranean Fever.—No official data were available with regard to temperature and rainfall for the whole of the period 1894 to 1903. The curves in the accompanying chart are constructed from figures kindly supplied me by the Rev. Father J. F. Dobson, S.J., the result of observations made at St. Julian's, near Valetta. I have inserted also a curve representing the case incidence of Mediterranean Fever, for comparison. The last-named curve is based on figures taken from the civil official notification records (see next page).

It will be at once seen that there is a very close correspondence between the curve representing the temperature and that representing the number of cases. The rise of the latter curve follows that of the former at an interval of about one month, which would be approximately sufficient to allow for incubation and notification if the incidence of fever were directly dependent upon the temperature of the air. The temperature curve attains its maximum in July and continues high during August, after which it begins to drop. The case curve attains its maximum a month later, but, unlike the temperature curve, it at once commences to drop, so that it would appear that whatever connection the air temperature may have with case incidence, does not remain so obvious after the former has attained its maximum.

The curve representing rainfall is in general the inverse of that representing temperature. It attains its minimum in July, but it is almost as low in June and August. The "case" curve commences to drop at the same time that the rainfall curve commences to rise, allowing no interval for incubation and notification, so that the connection is not clear; nor does the steep rise of the rainfall curve, at the end of September, produce a correspondingly steep decline in the case curve as might have been expected were the connection between the two intimate.

Seasonal Prevalence of Mediterranean Fever.—On p. 37 will be found a table giving the number of cases that were notified each month of



each year of the period 1894 to 1903, together with the total number of cases which were notified during the period in each month.

The figures in Table VI probably represent with some accuracy the seasonal prevalence of Mediterranean Fever, because though they are founded upon the civil official notification returns, there does not appear to be any particular reason why these returns should be more inaccurate at one time of the year than at another.

Table VI.

	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
1894	22	6	35	28	24	18	44	22	53	30	16	21
1895	10	9	21	23	11	26	16	47	42	18	27	12
1896	18	11	16	27	70	69	126	145	71	33	31	30
1897	28	25	22	30	52	50	92	89	40	47	41	38
1898	41	16	17	20	36	32	49	40	50	64	54	38
1899	61	33	49	39	47	63	105	77	58	103	91	71
1900	38	30	37	33	37	42	65	83	79	78	82	44
1901	46	32	25	24	41	67	74	89	78	64	43	42
1902	17	34	37	34	33	37	66	84	71	61	49	65
1903	32	26	31	23	35	54	68	82	54	71	33	44
Total	313	222	290	281	386	453	605	758	605	569	467	455

The maximum prevalence would appear to be in the month of August and the minimum in February. It is to be noted that the number of cases notified during February does not approach zero, being roughly 30 per cent. of the number notified in August.

From February to August there is each month a steady increase in the number of cases, except for a slight drop-back in April, and from August to February a steady decrease.

Consideration of Ways in which the Infection of Mediterranean Fever may be Transmitted.

(1) *Direct Personal Infection* (that is, by contact, or by the breath, or by the saliva).—No reliable data as to the number of dwellings in which more than one case of Mediterranean Fever occurred, could be obtained from the official notification records. The name of the patient was seldom given, and on visiting an address which had appeared more than once in the records, it was generally found to be a tenement house occupied by 15 or 20 families. Often there was no record of the number of the house, the street only being indicated.

Amongst 100 houses which I personally visited, in all of which Mediterranean Fever had been notified during 1904, I only found six houses in which there had been more than one case notified. There was often strong probability that other cases of a lighter nature than that notified had occurred, but the information elicited was never conclusive.

In Malta, outside Valetta, Cospicua, Vittoriosa, and Senglea, as noted above, both density of population upon area, and aggregation of a large number of persons in one locality appear to favour the spread of Mediterranean Fever. This may be because such conditions give greater opportunity for close personal contact, and so for direct personal infection. In making any inference it must not be forgotten

that Valetta and the three cities are exceptions, although both conditions are present in an eminent degree. It would appear, therefore, that whatever may be the conditions which favourably differentiate Valetta and the three cities from the rest of Malta, they must have an enhanced importance in their bearing upon the spread of Mediterranean Fever, inasmuch as they seem to more than counterbalance two conditions which appear to favour the spread of the disease in other parts of Malta.

Thirty-five women, wives of non-commissioned officers in the garrison, were attacked by Mediterranean Fever during 1904. Only two of them were removed to hospital. If direct personal infection were always an important factor in the spread of Mediterranean Fever, it would be expected that a large proportion of the husbands of these women would have been attacked, yet only five fell ill. Moreover, in two cases out of the five the husband and wife appear to have fallen ill on the same day, which would point rather to infection from a common source, than to infection from husband to wife, or *vice versa*.

Set out in the following table is a comparison between the three principal hospitals of Malta, showing separately the incidence of Mediterranean Fever upon the nursing staffs, and upon the patients undergoing treatment in hospital for other diseases, during the five years 1899 to 1903 respectively. I have added to the table, for comparison, the incidence of Mediterranean Fever upon the troops quartered in Valetta during the same period.

The number of patients per 1000 constantly ill, in other words, per mean yearly number of patients in hospital, is not strictly comparable with that of the staff per 1000 of strength, because in the former we are dealing with a shifting population in which fresh patients would constantly become exposed to infection, if the hospital were a centre of infection, whereas the staff would not vary in the same degree. In addition, the patients in hospital would be more numerous in summer, owing to "simple continued fever," than in winter.

It will be seen that during the period the average incidence of Mediterranean Fever upon patients treated in the Station Hospital, Valetta, was less than that upon the troops quartered in Valetta, while in the other two hospitals it was much greater. In all three hospitals very severe incidence occurred on the nursing staffs.

For the better consideration of the difference of incidence upon the respective staffs of the three hospitals I add here a short note upon each.

The Station Hospital, Valetta, has only attempted to isolate Mediterranean Fever since 1903. The isolation is incomplete; the Mediterranean Fever wards are separated from other wards only by a wall reaching about half-way to the ceiling. Enteric cases are frequently put in the Mediterranean Fever wards, and I have seen at least one case of Mediterranean Fever in a general ward.

Table VII.

	Valeta Station Hospital.			Central Civil Hospital.			R. N. Hospital, Bighi.			Valeta troops.
	No. of cases of Mediteranean Fever admitted.	No. of cases of Mediteranean Fever which occurred amongst hospital patients per 1000 constantly ill.*	No. of cases of Mediteranean Fever which occurred amongst hospital orderlies per 1000 of strength.	No. of cases of Mediteranean Fever admitted.	No. of cases of Mediteranean Fever which occurred amongst hospital patients per 1000 constantly ill.*	No. of cases of Mediteranean Fever which occurred amongst hospital nursing staff per 1000 of strength.	No. of cases of Mediteranean Fever admitted.	No. of cases of Mediteranean Fever which occurred amongst hospital patients per 1000 constantly ill.*	No. of cases of Mediteranean Fever which occurred amongst sick bay staff per 1000 of strength.	
1899..	84	32·00	34·48	129	58·44	171·4	111	66·67	83·3	22·54
1900..	85	6·44	54·08	147	100·63	171·4	261	89·55	459·5	26·18
1901..	127	45·75	121·21	127	96·15	188·9	175	78·43	122·0	48·11
1902..	60	34·18	48·78	127	47·62	111·1	272	175·60	476·2	16·90
1903..	222	12·26	50·00	154	83·80	83·2	279	102·74	150·3	17·81
		25·9	56·5	—	77·2	184·8	—	105·18	289·34	34·81

* The number of cases which occurred amongst hospital patients was calculated for each hospital by reckoning all cases of Mediterranean Fever which occurred in patients more than 14 days after their admission, and less than 14 days after their discharge. Patients who were admitted suffering from a disease that might have been Mediterranean Fever are excluded.

The means provided for the disposal of bed-pan contents and slops are wholly inadequate. A separate slop sink is used for enteric and Mediterranean Fever cases only. It is, however, on a different landing to the wards, and at a considerable distance. It is not supplied with a proper flush for cleansing purposes, and it is in a small dark room with faulty pavement. Some bed-pans examined by me were found to be very foul, though supposed to be cleansed. Izal is used for disinfecting the bed-pans, and carbolic solution for the orderlies' hands. The patients are cleansed with carbolic solution after the bed-pan has been used, and izal is put in the bed-pan before use.

Water-closets are used by enteric and Mediterranean Fever convalescents in common with other convalescents. The urinal provided is of the perpendicular slab type with an insufficient flush. It smelt offensively and the floor was soiled with urine.

The hospital orderlies spend about 50 hours per week in the wards.

The Royal Naval Hospital, Bighi, has isolated Mediterranean Fever for the past three years in special wards. These wards are amply provided with modern hospital sinks containing powerful flushes. Three of the sinks in the hospital were not ready for immediate use at the time of my visit. Izal is placed in the bed-pan before use, and an india-rubber sheet is put under the patient. The sheet shown me was perished and soiled. The patient is cleaned with soap and water, but the attendant does not disinfect his hands as a routine matter.

The sick-bay staff spend between 66 and 71 hours in the wards per week. Soiled linen is conveyed to the laundry by the sick-bay staff and washed with the other linen.

The Central Civil Hospital.—No attempt is made here to isolate patients suffering from Mediterranean Fever. They are distributed at haphazard throughout the medical wards.

Bed-pans are emptied into a gully in an open space between the wards. The edges of the gully are protected by a metal funnel, but the bed-pans are carried carelessly across the open space, dripping portions of their contents on the pavement, and are roughly washed out at a hot-water tap over an ordinary grating.

The attendant does not cleanse his hands after the operation, and the bed-pan is not usually disinfected, though occasionally a small portion of "carbolic" powder is dusted into it. No attempt was made to cleanse the patient at the time of my visit, and his person and bed proved upon inspection to be in a filthy condition.

Infected clothing is said to be steeped in tubs containing a 1 in 1000 solution of corrosive sublimate before removal to the laundry at the poor-house. At the time of my visit there was no infected clothing in the two small tubs shown me, and I was informed that the liquid they contained was not corrosive sublimate solution.

The nurses spend practically all their time in the wards, eating

and sleeping there; but they have a holiday every third day from midday until 6 the next morning, or if it happen to be a visiting day (Wednesday or Sunday), they leave at 4 P.M. instead of midday. In addition, they have a holiday every three weeks from 9 A.M. to 6 A.M. the following day.

Male ward cleaners have the same hours except that they leave hospital on alternate days at 5 P.M., returning at 6 A.M., and on alternate Sundays from 8 A.M. to 6 A.M. on Monday.

Briefly, the Military Station Hospital has practised partial isolation during 1903.

The Royal Naval Hospital has practised complete isolation during the past three years.

The Central Civil Hospital has made no attempt at isolation.

The Military Hospital and the Naval Hospital take precautions against the spread of infection by excreta; while in the Civil Hospital such precautions are almost altogether neglected.

The incidence upon the staffs of the three hospitals is not in proportion to the precautions taken against infection, nor to the number of hours spent in the wards.

The patients and attendants in the civil hospital are entirely Maltese, and if the incidence upon either the one or the other be compared with the incidence upon the civil population of Malta as a whole, the result is remarkable. The attendants show an incidence of 134.8 per 1000, the patients 77.2 per 1000 constantly ill, while the civil population of Malta shows only 3.2 per 1000 during the 10 years, 1894 to 1903. These results are no doubt due largely to faulty notification.

It is possible that so-called endemic acquired immunity may play a part in reducing the incidence on the patients and attendants in the civil hospital, and that such immunity may invalidate comparisons with hospitals occupied altogether by Englishmen.

If the Valetta Station Hospital be compared with the Naval Hospital it will be found that the incidence of Mediterranean Fever upon the respective staffs and patients is not in proportion to the amount of isolation attempted, but is more or less in proportion to the amount of care exercised in disposing of the excreta of patients, and the number of hours spent in the wards by the attendants.

The cause of special incidence of Mediterranean Fever in the hospitals does not, on the evidence obtained, appear to be direct personal infection, since that would probably be more evident in the hospital where least isolation is attempted. The incidence, mentioned above, on non-commissioned officers whose wives suffered from Mediterranean Fever points in the same direction. Neither is the evidence in favour of a place infection, to which the patients would probably be more exposed

than the staff, and would, in addition, constantly present fresh material.

It is possible that an aggregation of cases of Mediterranean Fever in one place may be more infective than the same number spread over a large area, but we have no evidence to point to this. In the Naval Hospital during 1904—a very short period, no doubt—only one case of Mediterranean Fever arose amongst the patients occupying the adjoining wards to the Mediterranean Fever wards. A table showing the cases of Mediterranean Fever which occurred amongst patients in the Naval Hospital during 1904 will be found on p. 49.

(2) *Excretal infection* may occur by means of infected dust inhaled or swallowed, by contamination of the hands, and thence the mouth, or by contamination of food or drink.

Dust is very prevalent out of doors in Malta, because of the friable nature of the rock and the hot sun, and owing to the high winds prevalent, opportunity of inhaling or swallowing it is present during the greater part of the year. There is, without doubt, possibility of frequent pollution of the dust by the excreta of mild or unrecognised cases, more especially in the suburban area, where conveniences are few and scavenging desultory. Horrocks has shown that the *Micrococcus melitensis* is found in the urine of Mediterranean Fever patients, and that it can live for long periods in a desiccated condition.

The seasonal curves of temperature and rainfall are such that the degree of dust prevalence corresponds closely with the degree of Mediterranean Fever prevalence. It is true that the Mediterranean Fever curve begins to fall immediately after attaining its maximum, while the curve of temperature remains high and the curve of rainfall low; but that may be accounted for by the fact that the "Sciroc" wind begins to blow in August. I am informed that this wind is so laden with moisture that it renders the roads damp during its prevalence in August and September. I cannot say I observed this phenomenon during my stay in Malta, but during the summer of 1904 there was very little "Sciroc," although the temperature was unusually high.

The rainfall curve is on the whole consistent with the theory of dust infection, being in general the inverse of the Mediterranean Fever curve; but I understand that during long periods in the rainy season there is little or no dust. If this be so, and dust be largely concerned in the spread of infection, it would be expected that there would be corresponding periods almost free from Mediterranean Fever notifications, which is not the case.

I have made out the following table with a view to comparing the incidence during the most dusty months of the period 1894 to 1903 with that during the least dusty months, for the three areas, urban, suburban, and rural.

	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Urban area—												
Valetta.....	19	14	30	12	20	23	63	40	37	20	33	29
Floriana.....	15	5	16	16	14	36	33	40	29	26	23	17
Coopious.....	8	8	4	7	4	45	38	28	16	21	10	8
Vittoriosa.....	8	7	6	3	7	20	15	23	23	21	12	14
Senglea.....	5	1	3	3	7	9	8	11	17	7	5	8
Suburban area—												
Mianda and Pietà.....	55	35	59	41	56	133	157	142	127	95	83	76
Sliena and St. Julian's.....	14	10	6	6	7	16	35	41	24	20	15	14
Hamrun.....	25	15	22	26	28	36	75	58	63	53	21	48
Birchirchara.....	30	26	35	38	43	48	63	117	63	69	44	33
Birchirchara.....	37	29	26	27	25	34	66	45	43	45	32	27
Curni.....	1	4	3	2	2	3	6	12	3	5	6	1
Tarxien and Paola.....	5	6	5	7	11	10	9	10	6	10	13	9
Zabbar.....	19	10	23	7	18	14	22	25	19	22	18	17
Rural area—												
Notabile and Rabato.....	131	100	120	113	134	159	276	308	226	224	149	149
Dingli.....	15	10	7	18	14	18	40	24	21	33	13	30
Zebbug.....	1	2	—	2	1	2	2	6	8	5	—	1
Siggei.....	16	12	15	16	25	12	28	46	37	31	30	22
Lia, Attard, and Balzan.....	3	3	6	3	5	5	14	12	11	8	5	11
Naxaro.....	10	3	12	28	37	19	49	50	37	42	24	19
Musta.....	6	6	4	1	7	8	9	13	17	14	15	9
Gargur.....	20	4	5	9	4	11	11	18	19	34	44	12
Melheha.....	—	—	1	9	—	2	2	5	6	5	8	2
Luca.....	1	1	—	1	1	2	—	4	2	6	6	2
Zurrico.....	—	—	1	4	1	2	2	2	3	2	4	5
Krendi.....	10	4	2	1	3	5	12	6	14	12	5	13
Safi.....	—	—	1	2	4	1	4	6	3	2	2	6
Micabiba.....	1	3	—	—	1	1	1	2	—	1	1	—
Chircop.....	1	1	—	—	1	—	2	5	3	3	2	5
Zeitun.....	—	1	—	—	1	—	2	3	2	2	—	2
Aziak.....	13	10	13	13	24	10	32	31	26	9	25	16
Gudja.....	4	1	1	1	1	4	3	3	8	1	3	3
	3	—	2	1	1	4	8	4	2	3	1	3
TOTAL	104	61	70	100	131	107	221	240	219	212	188	180

I take March to September inclusive as being the driest and consequently the most dusty months; and January, February, October, November, and December as the wettest and least dusty months. Allowing a month for incubation and notification, we have April to October representing the dry part of the year and the remaining months the wet.

In the urban area the average number of cases in the dry season was 10·73 per month, and in the wet season 6·16; that is in the proportion of 100 to 56.

In like manner in the suburban area the average number of cases per month in the dry season bears to the average number of cases per month in the wet season the proportion of 100 to 63, while a similar comparison in the rural area gives a proportion of 100 to 66.

	Proportion of cases per month in the wet season to the cases per month in the dry season.
Urban area.....	100 to 56
Suburban area	100 „ 63
Rural area	100 „ 66

This is not the result to be expected had contaminated dust contributed largely to the spread of Mediterranean Fever. There is less dust in the urban area, and less opportunity for dust contamination, on account of superior paving, draining, and scavenging, and also because all the urban area abuts upon the sea, much of it being built on tongues of land almost surrounded by water. It would be expected that the difference between the prevalence of Mediterranean Fever in the dusty season and in the wet season would prove least marked in the urban area, but, on the contrary, the difference is most marked in this area. These figures, taken for what they are worth, do not indicate that contaminated dust in the open air has a marked influence upon the incidence of Mediterranean Fever.

In the consideration of excretal infection by way of the hands or by way of food, special incidence upon certain hospital nurses and orderlies who have the handling and cleansing of Mediterranean Fever patients has already been noticed under the heading "Direct Personal Infection."

Abundant opportunity for soiling the hands and for pollution of the food is afforded by the methods of excreta disposal in use in the islands, which have already been referred to in Part I of this report. Amongst 100 houses examined in which Mediterranean Fever had occurred during 1904, I found that 75 had faults of one kind or another which rendered pollution of the hands, or of food, with excretal matter, probable. Amongst 40 other houses not infected with

Mediterranean Fever during 1904, but examined by way of control, I found 55 per cent. suffering from faults of a like kind. The control houses were selected by reason of their similarity to the infected houses; they were very often next door, and they were always of the same class and in the same neighbourhood as the infected houses. Large figures, however, extending over several years, would be required to give value to such data as these. Such as it is, however, the evidence is in favour of the probability of excretal pollution of the hands or food, or of dust inside houses having played a part in the spread of infection. As against this probability there is the fact that amongst the civil population, where opportunity for this kind of infection is far greater than amongst the garrison or Navy, the case incidence of Mediterranean Fever is in general about one-eighth as severe. Here, however, the notification returns are probably at fault.

(3) *Newly-turned earth* has been suspected by more than one observer to be a cause of outbreaks of Mediterranean Fever. When the porous nature of the rock in Malta is considered together with the fact that sewage has been allowed to percolate into it, and into the soil above it, for centuries, it does not seem remarkable that digging operations should have been suspected.

Mr. Cartwright-Read, the Admiralty Superintendent of Works, kindly undertook to furnish me with immediate notice of cases of sickness arising amongst the men employed by him on digging operations in Fort St. Angelo during my stay in Malta. In July 255 men were employed, in August 327, and in September 337. All men absent from work on account of illness during these three months were visited and reported upon, and no case of Mediterranean Fever was discovered. The ground opened up was probably at one time very much fouled, part of it having been the ancient prison of the galley slaves employed by the Knights of St. John.

The Honourable Mr. Gatt, Superintendent of Public Works to the Maltese Government, kindly placed similar facilities at my disposal with regard to gangs of men at work laying sewers during July, August, and September of 1904. The numbers of men employed were 200, 230, and 310, in each month respectively. No case of Mediterranean Fever was detected amongst them.

It appeared to me possible that the men at work in sewer laying had attained a certain immunity from infection, such as is said to be acquired by sewer men at home, and that the opening of the earth might have had a deleterious effect upon the health of the occupants of the houses in the localities where the sewers were being laid.

Curmi, Misida, and Sliema have been sewered during the last few years, the actual period occupied in laying sewers being for Curmi, March, 1901, to October, 1901; for Misida, the whole of 1903; and

for Sliema, November, 1901, to October, 1902. Contrasted with the five-year period 1899 to 1903 there was, as shown below, a slightly greater incidence of Mediterranean Fever in Sliema, and a considerably smaller incidence in Misida during the period that sewers were being laid. In Curmi the incidence was about three times as severe during the laying of the sewers as it was during the five-year period, but here we are dealing with only a very small number of cases. On the whole, the figures below do not indicate that opening streets to lay sewers has any marked effect in increasing the prevalence of Mediterranean Fever.

Table IX.

	I. Period during which work was in progress.	II. Average number of cases of Mediterranean Fever per year per 10,000 inhabitants.	
		During the period set out in Column I.	During the period 1899—1903.
Sliema	Nov., 1901—Oct., 1902	46·9	43·3
Misida	1903	74·4	83·6
Curmi	Mar.—Oct., 1901.....	27·6	9·5

(4) *Biting Insects*.—Further investigation is necessary before any definite pronouncement can be made as to the part, if any, taken by biting insects in the spread of Mediterranean Fever. Up to the present little is known as to the life history, distribution, and seasonal habits of even the commoner biting insects found in the Maltese Islands. I have, for instance, evidence that mosquitoes bite in the winter in Malta, but I do not know what kind of mosquitoes do so. The sand fly is very prevalent in parts of Malta during the summer, but there is little information as to his breeding places or his time of flight, or his winter habits.

The researches of Shaw and Gilmour would show that it must be a matter of some difficulty for a biting insect to infect himself from the human subject, seeing the sparse numbers in which the *Micrococcus* has been found in the peripheral circulation, and the small amount of blood the insect is capable of taking. Similarly, the chances would be infinitesimal of an insect carrying even a single *Micrococcus melitensis* mechanically upon his proboscis. On the other hand, it may be said that if certain biting insects, like mosquitoes, were capable of infecting themselves with Mediterranean Fever and of transferring the infection, the disease would be much more prevalent than it is.

The special incidence upon hospital orderlies, in comparison with hospital patients, is against the biting insect theory. The patients would be more likely to get bitten by infected insects than would hospital orderlies, both on account of being constantly in the wards and because they are less able to defend themselves.

(5) *Water* does not appear to have played any considerable part as a carrier of Mediterranean Fever infection in Malta. The public water supply, which is reasonably free from suspicion of contamination, is laid on to every village in Malta except Mellieha, but most householders have an alternative supply in the shape of a rain-water tank, usually open to contamination. Generally speaking, the public water supply is not laid on to the houses, but is fetched from a stand-pipe, and it is apparent that there is often opportunity for contamination in process of transit, or on account of the place where the water is kept. I personally inspected 100 houses in which Mediterranean Fever had occurred during 1904, and as far as I could judge, 35 of them had a water supply that was not reasonably liable to contamination. Out of 40 houses inspected by way of control, 27 per cent. had a water supply which was not reasonably liable to contamination. In the same two groups of houses, 85 of the infected class used the public water service, while only 65 per cent. of the control class used it.

A comparison between the figures relating to enteric fever, often a water borne disease, and the figures relating to Mediterranean Fever, shows little correspondence in the distribution of the two diseases. For example, the average number of cases of enteric fever per 10,000 inhabitants in Malta per year of the period 1894 to 1903 was 7·2, and the incidence on the three areas referred to previously was as follows:—

Urban area.....	7·5 per 10,000 inhabitants per annum		
Suburban area	6·6	„	„
Rural area	6·9	„	„

I do not profess to account for this distribution of enteric fever, but it is obviously entirely different from that of Mediterranean Fever.

No connection has ever been demonstrated between any particular branch of the public water supply, nor between any particular well or tank, and an outbreak of Mediterranean Fever, though such connection has frequently been shown with outbreaks of enteric fever in Malta.

Aërated waters are much drunk in the summer time in Malta, but here the question is still one of water. The manufacture is carried on under Government inspection; the water used is generally the public water supply, and in some cases distilled water. No connection has ever been traced between aërated waters and Mediterranean

Fever; indeed, the majority of rural dwellers who are attacked by Mediterranean Fever seldom take aerated waters. The urban and suburban areas, where aerated waters are most taken, do not show a higher incidence of the disease than the rural area.

(6) *Milk* is not so closely connected with water in Malta as it is in most other countries, because the great majority of people get their milk supply in their own vessels direct from the goat.

My inquiries as to the precise source of milk supply in the houses I visited seldom elicited definite information; many of the persons interrogated did not know whose goats supplied them, being in the habit of hailing the first goat herd who passed the door.

More particular inquiries as to sources of milk supply in relation with Mediterranean Fever are now being made immediately upon notification, and when the results are tabulated at the end of July, 1905, some definite pronouncement may be possible.

(7) *Uncooked Foods*.—Inquiry was made in a large number of cases with regard to fruits and vegetables, or salads, but nothing tending to incriminate these articles of diet was elicited.

(8) *Infection by Cuts or Abrasions*.—No connection was established between breaches of continuity in the skin and subsequent attacks of Mediterranean Fever. Practically none of the cases I saw had a history of cuts or other abrasions. The only evidence that seems to point in the direction of infection of this kind is the severe incidence of Mediterranean Fever upon patients in the operation ward of the Royal Naval Hospital, Bighi, during 1904, a point which requires further investigation.

The following table gives some idea of the arrangement of the Royal Naval Hospital at Bighi, and the number of patients who developed Mediterranean Fever during 1904, more than 14 days after admission, or less than 14 days after discharge from the hospital.

The chief contributory wards were: C, the operation ward, and D, one of the suppuration wards. Cases were transferred from C to D, if suppuration supervened, and cases were also transferred, when necessary, from D to C. During 1904 seven cases were transferred from C to D, and five from D to C, so that the two wards were in a measure connected with one another. Only one case occurred in E3 or E4, which adjoin the Mediterranean Fever wards and open into them.

	Jan.		Feb.		Mar.		Apr.		May.		June.		July.		Aug.		Sept.		Oct.		Nov.		Dec.		Total.		
	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	Admissions.	Attacks.	
SURGICAL.																											
North block—																											
A (basement), venereal	15	—	10	—	8	—	13	—	27	—	19	—	20	—	8	1	9	—	13	—	21	—	1	1	164	2	
C (1st floor), operations	10	1	5	4	8	—	27	—	3	—	—	—	6	—	3	—	3	—	15	1	18	—	—	1	98	7	
E (2nd floor), suppuration	8	1	10	—	8	—	—	2	20	—	18	—	13	—	1	—	1	—	—	—	—	—	—	—	79	3	
South block—																											
B (basement), venereal	25	—	11	1	11	—	28	1	—	—	—	—	35	—	3	—	—	—	21	1	20	2	3	1	157	6	
B2 (basement), ophthalmic	5	—	5	—	1	—	2	—	10	—	4	—	4	—	—	—	—	—	3	—	1	—	1	1	36	1	
D (1st floor), suppuration	7	1	8	2	10	—	16	1	7	—	—	—	11	—	7	—	6	—	22	—	19	6	2	2	115	12	
F (2nd floor), suppuration	8	—	—	—	—	—	18	—	12	—	10	—	7	—	—	—	—	—	—	—	5	—	3	—	65	0	
Officers' cabins	4	—	1	—	4	—	6	—	4	—	35	—	2	—	—	—	—	—	4	—	9	—	1	—	70	0	
MEDICAL.																											
East block—																											
1. Mediterranean Fever	—	—	1	—	1	—	2	—	—	—	—	—	2	—	19	—	8	—	25	—	11	—	2	—	71	0	
2. Mediterranean Fever	1	—	1	—	1	—	8	—	7	—	8	—	7	—	10	—	—	—	15	—	1	—	—	—	59	0	
3. Enteric and scabies	7	—	5	—	4	—	9	—	2	—	—	—	3	—	—	—	—	12	—	12	—	1	—	—	55	0	
4. Zymoties	—	—	—	—	8	—	4	—	2	—	3	—	3	—	1	—	—	—	3	—	—	—	1	—	24	1	
Officers' cabins	10	—	9	—	12	—	13	—	11	—	6	—	14	—	10	—	6	—	17	—	4	—	1	—	113	0	
West block—																											
1. General medical	42	—	56	—	43	—	51	—	35	—	27	—	62	—	18	—	10	—	45	—	40	2	7	—	436	3	
2. General medical	1	—	—	—	6	—	17	—	16	—	—	—	25	—	—	—	—	—	—	—	—	—	—	—	65	0	
3. Tubercle	5	1	1	—	9	—	1	—	2	—	—	—	—	—	6	—	—	—	9	—	2	—	—	—	35	1	
4. Tubercle	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Officers' cabins	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

PART III.—OUTBREAK OF MEDITERRANEAN FEVER IN THE 2ND
BATTALION OF THE ESSEX REGIMENT.

This was the only definite outbreak of Mediterranean Fever that I had an opportunity to observe personally, during my stay in Malta.

The 2nd Battalion of the Essex Regiment arrived at Malta from England on April 29, 1904. With the exception of some details sent to Gozo, the regiment was quartered at Lower St. Elmo Barracks, in Valetta until May 19th, when it went to Pembroke Camp for musketry. It remained at Pembroke Camp until May 28th, and then went on to the camp at Mellieha, whence it returned to Lower St. Elmo on June 10th. It was still at the last-named barracks at the end of September.

The first known cases of Mediterranean Fever in the battalion occurred on June 10th, when two sergeants fell ill, another went sick on the 11th, a corporal and a sergeant on the 14th, and so the cases kept dropping in until, on August 27th, there had been 35 cases, 21 of which were directly connected with the sergeants' mess.

The type of the disease was very severe. Cases 4, 9, and 13 died and others were still in danger at the end of September.

Table XI shows the number of known cases of Mediterranean Fever in the Essex Regiment and the date of onset of the disease in each instance.

Of these 35 cases, occurring in $2\frac{1}{2}$ months, 21 were directly connected with the sergeants' mess; as regards the remaining 14, indicated in the table by a star, there does not appear to have been such connection except in the case of No. 12A. This man, however, returned to Gozo on June 10 to find his wife and child sickening with Mediterranean Fever. The probability of his having been infected by his wife or child, or from the same source as they were, seems stronger than the probability of his having been infected while with the regiment. I have therefore not included him amongst the cases likely to have been infected at the sergeants' mess.

Case 20 was in hospital with simple continued fever during most of the regiment's stay at Pembroke, and he returned to duty at St. Elmo instead of going to Mellieha. He left St. Elmo before the regiment's return, and went to Ghain Tuffieha Camp, and from there to Gozo, where he was attacked by Mediterranean Fever. His illness has therefore no causal relation with the main part of the regiment.

The 21 cases connected with the sergeants' mess form a continuous series of 19 attacks in six weeks, with never more than three or four days' interval. After July 16 there is an interval of 11 days

Table XI.

No..	Date of onset.	Name.	Remarks.
1	June 10	Sergt. H.	
2..	" 10	" B.	
3..	" 11	" W.	
4..	" 14	Lance-Sergt. H.	
5..	" 14	Corpl. J.	Used to go into sergeants' mess for orders.
6..	" 17	Sergt. L.	
7..	" 20	Pte. M.	Waiter in sergeants' mess.
* 8..	" 21	" H.	No connection shown with sergeants' mess.
9..	" 23	Sergt. F.	
10..	" 23	Lance-Sergt. S.	
11..	" 24	" B.	
12..	" 25	" B.	
*12A	" 25	" W. ..	Wife and child had Mediterranean Fever at Gozo, where he rejoined them June 10.
13..	" 28	Y.	
*14 ..	" 29	Pte. F.	No connection shown with sergeants' mess.
15..	July 1	Sergt. P.	
16..	" 1	" F.	
17..	" 4	" M.	
18..	" 5	" V.	
19..	" 7	" K.	
*20..	" 8	Pte. P.	
21..	" 12	" M.	Waiter in " sergeants' " mess " until May 28, 1904.
*22..	" 15	" L.	No connection shown with sergeants' mess.
23..	" 16	Lance-Corpl. B.	Cleaned men's latrines; was often in sergeants' mess for odd jobs.
*24..	" 23	Pte. H.	No connection shown with sergeants' mess.
25..	" 27	" L.	Waiter in sergeants' mess.
26..	" 31	Sergt. H.	
*27..	Aug. 5	Lance-Corpl. S.	No connection shown with sergeants' mess.
*28..	" 10	Pte. W.	" " " "
*29..	" 13	" C.	" " " "
*30..	" 13	" C.	" " " "
*31..	" 14	" C.	" " " "
*32..	" 18	" S.	" " " "
*33..	" 26	" A.	" " " "
*34..	" 27	" W.	" " " "

without a fresh case, and then follows Case 25 on July 27, and four days later, on the 31st, Case 26. There were no other known cases among persons connected with the sergeants' mess up to the end of September, when I left Malta.

As nearly as could be ascertained there were about 60 persons connected with the sergeants' mess, while there were in all 616 men

and officers at Mellieha. Of the 60 persons, 21 were attacked by Mediterranean Fever, while of the remaining 556 persons, 14 only were attacked, and these for the most part at a later period than members of the mess in question.

A short description of the three places occupied by the regiment between April 29, the date of their arrival from England, and the end of September, is as follows:—

Pembroke Camp is the chief musketry camp for the island. It is situated a few miles north of Valetta on the sea shore. The camp is on rocky ground sloping to the sea. The rock is the common calcareous sandstone of Malta, and the intervals between the outcrops are filled by a sandy red loam, which easily pulverises and forms dust.

Mellieha Camp is situated on the bay of the same name, about half-a-mile north of the village of Mellieha, the most northerly village in Malta. The soil in and around the camp is loose and sandy, overlying the upper coralline limestone. The sergeants' mess, a wooden building, is on the edge of the camp, just above a steep sandy slope leading down to the sea.

Lower St. Elmo is a part of the fort which occupies the seaward end of the tongue of land on which Valetta is built. The barracks are below the level of the outer ramparts. The fort is built entirely on the calcareous sandstone.

Both at Pembroke and at Mellieha the regiment was under canvas. An examination of the situation of the tents in which persons attacked by Mediterranean Fever had slept, showed no considerable incidence on any one part of the camp more than on another.

At Pembroke the sergeants messed in a marquee which was equally exposed to the dust with the tents in which the privates lived and ate their food. The marquee stood upon similar soil to the tents, and it was subject to the same conditions with regard to proximity to latrines and urine tubs. It was no more liable to foul air emanations than the tents of the privates. At Pembroke the same latrines (dry earth) were used by the sergeants and by the privates, though a portion was reserved for the former. Complaints were made of offensive smell and of flies at the latrines.

At Mellieha the sergeants' mess hut stood some 40 yards away from the tents of the men. It was a wooden building, raised from the ground on posts, and protected from dust by windows. Built on to it were two water-closets, which were not used during the regiment's stay, because they were said to be out of order. These closets were directly connected with the sergeants' mess room by a louvred ventilator. I found after inquiry on the spot, that these water-closets had become unsealed owing to evaporation. It was therefore possible for sewer air to escape into the mess room from the whole length of sewer below the closets, and from the septic tank to which

this sewer conveyed the sewage of the camp. There were separate latrines (water carriage) for the sergeants and the privates at Mellieha; no complaint of smell or nuisance was made as regards either set of latrines.

At St. Elmo the sergeants' mess was situated on a site elevated some 30 feet above the barrack yard across which it faced the men's barrack rooms, at a distance of 40 or 50 yards. The mess was approached by a path seldom used for any other purpose, and the latrines were on the same level as the mess room, about 10 yards distant from it. I found no fault with these latrines other than the faults inherent to their pattern, which I have already discussed; but the latrines for privates in the same barracks were insufficiently flushed and most offensive.

No duty could be heard of which was likely to bring the sergeants of the regiment together into one place, except that of marking at the butts. (There were butts at Pembroke, but not at Mellieha, or St. Elmo.) There were, however, as many corporals and privates employed at the butts as sergeants, yet there was no special incidence of Mediterranean Fever on the former as there was on the latter.

The only places frequented by sergeants, but not by privates, were, at Mellieha and St. Elmo, the sergeants' mess and latrines, at Pembroke, and the sergeants' mess.

The sergeants who were attacked by Mediterranean Fever seem to have been infected roughly in proportion to the amount of time spent in the mess. The married sergeants would frequent the mess at Pembroke and Mellieha equally with the unmarried ones, while at St. Elmo they would spend more of their time at the married quarters. Twenty out of 51 sergeants who frequented the mess at all three places were married. Six of them were attacked before June 25, of whom three were married (the presumption being that cases before the 25th were infected at Mellieha or Pembroke), while of the remaining 12 cases, infected presumably at St. Elmo, only three were married. The number of cases before the 25th is, however, too small to allow of any great importance being attached to the increased incidence on married sergeants before that date.

Supposing, however, that the sergeants' mess, including, at Mellieha and St. Elmo, the sergeants' latrines, afforded the conditions for contracting infection, the question arises as to whether the mess at Pembroke, that at Mellieha, or that at St. Elmo, was chiefly concerned.

The first cases occurred on the 14th day after the regiment left Pembroke. This allows time for an incubation period consistent with the supposition that the disease was contracted at Mellieha, and to a corresponding extent tends to exculpate Pembroke, though it remains possible, of course, that the first few cases became infected at Pembroke. At Mellieha there were circumstances which differentiated the condi-

tions of life at the sergeants' mess from the conditions obtaining in the camp generally, but at Pembroke the same kind of difference did not exist. Some such differences as those obtaining at Mellieha deserve attention in attempting to account for the enormous excess of incidence upon persons connected with the sergeants' mess. Upon the whole, I think it is not likely that the sergeants' mess at Pembroke was seriously, if at all, concerned in the spread of the fever in the regiment, and that it is likely that the cause of the outbreak operated first of all at Mellieha. It would appear certain, however, that the sergeants' mess at St. Elmo had later on a share in spreading the infection, because if we were to suppose that infection was spread only at Mellieha, the average incubation period for Cases 13, 15, 16, 17, 18, and 19, would amount to at least 23 days, and to much more if Cases 21, 23, 25, and 26, were also referred to Mellieha. Although very long incubation periods have occasionally been reported, yet a succession of cases having so lengthened an incubation as the above, seems to me very improbable. I refer here only to the cases connected with the sergeants' mess, the other 13 cases occurring as they did most of them later on, and amongst over 500 men, did not show an incidence much greater than is to be expected under ordinary circumstances in Malta.

Food and Drink.—Bread, water, and milk came to sergeants and privates from the same sources. Butter, fruit, vegetables (salads, tomatoes, etc.), were eaten by both. Mineral waters were drunk by both, but those drunk by the sergeants were said to be made from distilled water, while those supplied to privates were not.

More precise inquiries were made amongst the members of the sergeants' mess and persons connected with it, including those of them who were ill with Mediterranean Fever.

Water.—Inquiry was made of 52 men connected with the sergeants' mess, including 19 of those attacked by the fever, as to water drinking. Cases 5 and 23 were not included because they neither ate nor drank in the sergeants' mess. Thirty-one men never drank water, and one other man only drank it on one occasion, and may for practical purposes be considered a non-water drinker. Of these 32, 11 were attacked (34·4 per cent.). Eleven men seldom drank it, and seven of these were attacked (63·6 per cent.). Nine men habitually drank water, and one was attacked (11·1 per cent.). Non-water drinkers then were attacked at the rate of 34 per cent., while water drinkers (habitual and occasional) were attacked at the rate of 40 per cent. On the other hand, occasional water drinkers were attacked nearly six times as severely as habitual water drinkers. The 11 occasional water drinkers drank it only when employed at the butts, when they obtained it from their water bottles. The habitual water drinkers also drank at the butts, in the same way, water from a like source.

Milk.—As result of inquiry made of 58 men connected with the sergeants' mess, it appeared that 3 drank unboiled milk by itself, and that 1 of them was attacked (33·3 per cent.); while of 55 men who did not drink unboiled milk by itself, 17 were attacked (30·9 per cent.). Case 4 was too ill to be interrogated, and is consequently excluded.

Milk with tea, coffee, or cocoa, was taken by all except three men. None of these three were attacked.

Mineral Waters.—Two out of 59 men interrogated did not drink mineral waters. One of these two was attacked.

Beer.—Of 59 men interrogated, it was ascertained that 52 took beer, and 17 of them were attacked (32·7 per cent.); while of 7 that did not take beer, 2 were attacked (28·6 per cent.).

Raw Vegetables (tomatoes, salads, etc.) were eaten habitually by 42 men out of 59, seldom by 9 men, and never by 8 men. Of the 51 who ate raw vegetables 14 were attacked (27·5 per cent.), while of the 8 men who did not eat them 5 were attacked (62·5 per cent.). Of the 14 cases among eaters of raw vegetables, 12 occurred among the habitual consumers and 2 among the occasional consumers.

Fruit.—Uncooked fruit was eaten habitually by 38 men out of 58, seldom by 13 men, and never by 7 men. Ten of the 38 who eat fruit habitually were attacked (26·3 per cent.). Four of the 13 men who seldom eat fruit were attacked (30·8 per cent.), and 5 out of the 7 who never ate it were attacked (71·1 per cent.).

Bread and Butter were partaken of by all from a like source.

It cannot be said that any one of the foods or drinks inquired about is incriminated by the above details, nor can there be much doubt that if any of the foods or drinks had been largely concerned in spreading the disease, the fact would have appeared. There is a slightly greater incidence upon those who drank water, but not sufficient, in face of the small numbers dealt with, to found any conclusion upon.

Bathing.—Inquiry was made as to bathing, because it has frequently been suggested that Mediterranean Fever might be caused by bathing in sewage-polluted waters. At Mellieha the sewage from the camp is discharged into the shallow waters of the bay, after passing through a septic tank, and at St. Elmo, situated as it is on the point separating the Grand and Quarantine harbours, there is considerable pollution of the water by sewage. Out of 64 men interrogated, 58 bathed and 19 were attacked (32·8 per cent.), while of 6 men who did not bathe, 2 were attacked (33·3 per cent.).

Personal Infection.—The question of direct personal infection from man to man was considered. This may have occurred in the mess-room, but if so, why did it not also occur outside from sergeants to men? unless, indeed, it happened that infection was largely conveyed from man to man by means of spray thrown into the air in the act of speaking or coughing. This method of infection would no doubt be favoured

by the still air of the mess room, and by the propinquity therein of the sergeants to one another during conversation; it would not be so likely to operate in the open air, nor perhaps in the tents or barrack rooms at night. If direct personal infection, other than through saliva, were the mode of infection in this outbreak, it would have better opportunity for taking effect in the tents and barrack rooms at night than in the mess room. Many of those attacked by Mediterranean Fever slept in tents with other persons not connected with the sergeants' mess. For instance, 7 privates slept in the tent with Case 12 at Mellieha, and 21 privates in the same room with him at St. Elmo. Most of the unmarried sergeants who fell ill at St. Elmo slept in bunks in the room with 20 or more men, yet none of these men were infected. Five cases altogether occurred in persons who had slept in the same tent or room with earlier cases, and 3 of the 5 were connected with the sergeants' mess.

Biting Insects.—In view of the theory put forward by Zammit, inquiry was made of 58 men connected with the sergeants' mess as to whether they had been bitten by mosquitoes or sand flies. It was considered at the time that the answers received would probably be a better index of the toughness or insensibility of the deponent's skin, than of the facts as they really were; nevertheless the results are given for what they are worth. Forty-three men were conscious of having been bitten, and 7 of them were attacked by Mediterranean Fever (16·3 per cent.); while of 19 who were not aware of having been bitten, 11 were attacked (57·9 per cent.). These figures may be claimed as unfavourable to the hypothesis that biting insects play a part in the transmission of Mediterranean Fever to man. On the other hand, it may be contended that those who were not aware of having been bitten had the more insensible skins, and hence were less likely to take precautions with a view to preventing the insects biting them, and, in consequence, were the more likely to have been bitten. Cases 4, 9, and 13 were too ill to answer this interrogation, and are consequently excluded.

If infection were conveyed by a biting insect, the insect, to fit in with the circumstances of this outbreak, would require to be one which did not bite in bright sunlight, nor in the dark. It was practically only in bright sunlight or in the dark that the sergeants mixed with the men, and if the insect were in the habit of biting under these conditions, the men would have been infected as well as the sergeants. Again, the insect must be one likely to confine itself strictly to one building or marquee, seldom or never wandering 50 yards away, for at Pembroke, Mellieha, and St. Elmo the tents or barrack rooms were within 50 yards of the sergeants' mess.

It may be asked how would the insect be likely to have become infected. Mellieha Camp was occupied from May 3 to June 9 by three different regiments, but no case of Mediterranean Fever is

known to have occurred amongst them during their stay or 14 days after. Neither were any cases reported from Mellieha village during this period. The possibility, however, of there having been unrecognised cases of Mediterranean Fever in one of these regiments, or in the Essex Regiment, cannot be disregarded.

It should be noted that non-commissioned officers would provide better opportunity for the spread of personal or insect-borne infection, than would private soldiers. The former are always loth to go to hospital, and usually defer reporting themselves sick until the last possible moment, while the latter generally report at once. In this outbreak, for instance, Case 6 did not go into hospital until July 15, although he became ill on June 17.

The only biting insect, of which I am aware, that comes at all near fulfilling the conditions which this outbreak seems to require is the female *Stegomyia fasciata*. There is, however, at present, wide divergence in the views of various observers as to her flight and habits of biting. It would be remarkable if so short a distance as 50 yards proved an insuperable barrier for a winged insect, even for one which, like the *Stegomyia*, is generally supposed not to wander far. Specimens of the *Stegomyia* were to be found at the time at Mellieha and St. Elmo, but they were also to be found at Valetta Station Hospital into which all the cases were removed. In this hospital Mediterranean Fever patients were separated from other patients only by a partition 9 or 10 feet high, in a ward more than 20 feet high, and mosquito nets were not in general use, and yet it does not appear that other patients became infected specially at that time.

Latrines in Connection with the Outbreak.—In view of Horrocks' discovery that the *Micrococcus melitensis* is excreted in the urine, and the possibility that it is also excreted in the fæces, the question arises, supposing that infection were spread by the sergeants' mess at Mellieha and St. Elmo, was it spread principally by way of the mess room, or by the latrines? The latter, it will be remembered, were separate from those of the privates both at Mellieha and at St. Elmo. I am not now considering the disused water-closets at Mellieha. Both the latrines and the mess room were used by everybody connected with the sergeants' mess, except Cases 5 and 23, so that it is difficult to find any evidence to incriminate the one place as against the other. It can be said, however, that it is difficult to imagine an infection inherent to the mess room yet not likely to be conveyed directly from man to man outside, while if the infection be supposed to be inherent to the latrines and of excretal origin, the difficulty is not nearly so great: infected dust, due to the pattern of the latrines, or infection of the hands and thence the mouth or nose for instance. Against the possibility of the latrines having been principally concerned in the spread of infection is the following negative evidence

which is not of sufficient weight to be at all conclusive:—One and the same man attended to the flushing and cleansing of the sergeants' latrines both at Mellieha and St. Elmo, and he was not attacked by Mediterranean Fever. Cases 5 and 23 did not use the sergeants' latrines. In addition it may be said that if the first few cases of the outbreak were infected at Pembroke, where the sergeants used a portion of the same latrines as the men, it would be expected that some of the men would have been infected if the latrines were the source of infection. I have, however, already said that I do not think it probable that the early cases were infected at Pembroke, and the man who attended to the sergeants' latrines may have been immune to Mediterranean Fever.*

With regard to the two water-closets through which it was possible that sewer air obtained access to the sergeants' mess at Mellieha, there appeared to be two ways in which they might have contributed to spread the fever: either by allowing infected dust to enter the mess room, or by allowing sewer air to enter, and thus weakening the natural tissue resistance by causing sore throat, or general loss of tone. None of those attacked by the fever, however, complained of sore throat previous to the onset of the fever. As to the infected dust theory, specimens were procured of the dust in the ventilator and on the closet pan, and were injected into monkeys by Horrocks without producing any ill effect. These specimens were, however, very minute, and were procured some weeks after the regiment left Mellieha. But supposing that infected dust from these closets, or from the latrines, were the cause of fever at Mellieha, what then caused the continuance of the outbreak at St. Elmo? Again, if the general tone of the men's health was lowered by the inhalation of sewer air in the mess room at Mellieha, and they were thus rendered specially liable to Mediterranean Fever, their health should have recovered its normal tone at St. Elmo, other conditions being equal, and the outbreak should have ceased, which was not the case.

Septic Infection.—Inquiry was made of those attacked as to whether they had suffered from cuts or boils, or other skin lesions, before the onset of the fever, but in no case was the reply in the affirmative.

Conclusion.—The available evidence is not such as to justify a definite

* "Carbolic" powder was used as a disinfectant in the latrines from the time of the regiment's arrival in Malta until May 18, when it was discontinued by order of the War Office, on the grounds that it was not a disinfectant but only a deodorant. Its use was resumed by the Essex Regiment on June 20. In view of any question arising as to a connection between the disuse of the "carbolic" powder and the outbreak of fever, a specimen of the powder was examined for me by Horrocks, and he found that a 10-per-cent. solution in urine failed to kill a culture of *M. melitensis* in one hour. I do not therefore think that the use, or disuse of the powder can have had any influence on the potentialities of the latrines to spread infection.

conclusion as to the manner of propagation of Mediterranean Fever in this outbreak.

The facts narrated, however, are not without value for the epidemiologist. If they cannot be held to warrant positive assertion of the transmission of the malady by a particular agency, they are at least of service in strongly suggesting the exclusion, in this instance, of certain possible factors, and as regards other such factors, in affording means of considering the relative degrees of probability of their having been concerned with the incidence of the fever.

Regarded in this light, the evidence may fairly be held to indicate that articles of food and drink played no appreciable part in the dissemination of the disease. A like inference is justified concerning the possible influence of conditions associated with bathing, and with inhalation or swallowing of infected dust in the open.

The possibility of the fever having been conveyed by biting insects cannot so readily be dismissed. But a careful review of the facts and conditions adduced under this head does not favour acceptance of the hypothesis that the explanation of this outbreak is to be found in this direction.

The facts reviewed in this report under the heading of direct personal infection are only such as would suggest the transmission of the fever by this agency if the manner of transmission in this instance had been such as almost entirely to limit its operation to the sergeants' mess. I am not, so far, in possession of any facts on the bacteriological side capable of strengthening, or negating the possibility of transmission of the fever by saliva. I do not know even that the *Micrococcus melitensis* exists in the saliva of patients. It may be said, however, that infection by means of saliva affords a solution of the problem of transmission not inconsistent with the facts in this outbreak, so far as I have been able to discover them; but it must be added that other facts noticed in Part II of this report under the heading "Direct Personal Infection," do not point to the probability of saliva spray having played a part in the spread of infection. (See p. 37 *et seq.*)

There remain for consideration the possibilities of the fever having been transmitted by conditions other than direct personal infection, or conveyance of the disease by biting insects, associated with the latrines of the sergeants' mess, or with the sergeants' mess itself.

Hypothesis that Mediterranean Fever may be a "filth disease," and that the latrines in question became and remained for some time infected by *Micrococcus melitensis*, passed in the urine or fæces of persons using them, would point rather to the latrines having had relation with propagation of the disease than to like relation of the mess itself. Such hypothesis, however, involves considerations that require further investigation, and, without more complete knowledge than is now available, can be no more than tentative.

Besides the condition suggested by this hypothesis, there may be others, at present unknown, which future epidemiological investigation, combined with further acquaintance with the life history and habits of the specific contagion of the malady, may serve to reveal, and which may be found to explain, as regards this outbreak of Mediterranean Fever, the special incidence of the disease upon persons frequenting the sergeants' mess or using their latrines.

These inferences, and the relative degree of probability of each, have relation solely to this particular outbreak of Mediterranean Fever. Even did the evidence point conclusively to one particular agency as being solely concerned with the outbreak, it would not necessarily follow therefrom that such agency would have to be regarded as the only one having concern with the transmission of Mediterranean Fever generally.

PART IV.—GENERAL SUMMARY AND CONCLUSION.

Hampered by a sense of the inaccuracy of the civil notification returns, I have only attempted to draw the most general conclusions from them, except with regard to the seasonal incidence.

The evidence I have been able to collect is not sufficient to lead to any final conclusion. I hope, however, that I have been able to indicate in the course of Part II some directions in which further epidemiological investigations would be likely to prove profitable.

The distribution of Mediterranean Fever amongst the civil population goes to show that, outside certain paved and drained areas, aggregation of persons in one locality, and density of population upon area in a district, favour the spread of the disease. The distribution amongst the garrison depends mainly on the age of the men and their length of service in Malta, new arrivals and young men being more frequently attacked. As regards the Navy, I have only been able to obtain figures for three years. So far as they go, they tend to show that, when a ship is invaded in one year, it is also invaded in each successive year, if it remain on the station.

The incubation period seems, on the data I have been able to collect, to be about 14 days, but further evidence is necessary before a definite conclusion can be reached.

As to the mode of entry of the specific infection into the human body, the facts do not permit of a definite pronouncement. The evidence, so far as it goes, seems to show that food and drink have no marked connection with the spread of the fever. Newly turned earth falls into a like category.

As a whole, the facts do not indicate that dust infection, outside dwellings, or direct personal infection by contact, breath, or saliva, plays an important part in spread of the disease, but there is not evidence to justify the exclusion of any of these factors.

I have been able to collect little evidence either for or against the carriage of infection by biting insects.

The facts with regard to infection by means of excretal pollution of the hands, the food or the dust in houses, so far as I have been able to deal with them, are suspicious, but they are not sufficiently strong to justify any conclusion.

Some reform of the notification system in Malta is necessary before epidemiological investigation can be expected to produce the best results. In addition, facts must be collected and recorded immediately after their occurrence by competent observers. Such work cannot be adequately performed by the sanitary inspectors as at present trained in Malta.

I have endeavoured to provide for the immediate record of a certain number of facts in relation to cases of Mediterranean Fever during the year ending July 31, 1905, amongst the civil population, and in the Services. With regard to the former, I fear that laxity of notification will prove a stumbling block. I regret that an urgent invitation to the Maltese medical men to forward blood samples to the public health laboratory for confirmation, did not meet with the response I expected.

I hope, however, that the facts now being recorded may prove useful in the consideration of some points.

In the meantime, I am still in process of receiving information from Malta which I have requested, as I found it necessary, and I should prefer to await its arrival and the consideration of the facts for the year ending July 31, 1905, before making any recommendations.

I have to thank the following gentlemen for much help and information given me in the course of this inquiry :—Deputy-Inspector Cox, R.N.; Fleet-Surgeon Bassett-Smith, R.N.; Staff-Surgeon Gilmour, R.N.; Colonel Wolesley, R.A.M.C.; Lieutenant-Colonel Rhodes, R.A.M.C.; Captain Kennedy, R.A.M.C.; Lieutenant-Colonel Adair, R.E.; Lieutenant-Colonel Winter, Director of Supply and Transport; and Major Boyce, D.S.O.; the Honourable A. Gatt, Superintendent of Public Works; the Honourable A. Micallef, Comptroller of Charitable Institutions; Mr. Cartwright-Reed, Admiralty Superintendent of Works; the Rev. Father Dobson, S.J., and the medical officers of health of Malta and Gozo.

ON THE SAPROPHYTIC LIFE OF THE *MICROCOCCUS MELITENSIS*.

By Fleet-Surgeon P. W. BASSETT-SMITH, R.N.

In compliance with suggestions made at the Sub-Committee Meeting of November 27, 1904, I have, at Haslar, carried out independently, during the last three months, some experiments relating to the vitality of the *M. melitensis* outside the body, with special reference to the infection of the soil, clothing, sea and tap-water, through the agency of infected urine. During this time I have not myself been able to isolate the *M. melitensis* from the urine of any clinical case in the wards (most of them being chronic, with relapses), though it was often present in the peripheral blood, and have, therefore, had to employ urine artificially infected; from the experiments it was apparent that the grosser the infection the longer could the organism be recovered from the material infected.

Some check experiments were also made with broth cultures as a means of infection, though in all cases these had been previously cultured for a certain time in human urine, which apparently did not decrease the vitality of the *M. melitensis*.

In proving the results of these experiments, as to purity of cultures, the following tests were carried out:—

1. Microscopical examination for morphological characters.
2. Alkaline reaction with litmus broth and milk.
3. Inability to stain by Gram.
4. Reaction with known serum of a Mediterranean Fever case.

Vitality of the M. melitensis in Urine.

Series 1.—The urine employed was that taken from Mediterranean Fever cases, which had been proved not to contain the *M. melitensis*, and had been sterilised on three days, but not otherwise treated.

No. 1.—A considerable quantity of a surface culture on agar of *M. melitensis*, originally obtained from Netley, in 1901, was emulsified with 10 c.c. of sterilised urine from a Mediterranean Fever case. It was kept at a temperature of 22° C.

This was subcultured successfully daily up to the 18th, after which it was not recovered.

No. 2.—Equal quantities of a five day old broth culture of *M. melitensis* and sterilised Mediterranean Fever urine, were mixed and subcultured daily on agar. The organisms gradually died out, and were last detected on the 14th day.

No. 3.—Strongly alkaline urine was infected from an agar culture of *M. melitensis* of three days' growth. Here the organisms more quickly died out, being last recovered on the 12th day.

No. 4.—Sterilised urine of a Mediterranean Fever case was infected from an agar culture, which had been previously passed through urine and sea water. In this case it was not recovered from the urine later than the 9th day. The urine was then very alkaline, equal to standard deci-normal soda sol.

No. 5.—Very slightly alkaline sterilised urine of a Mediterranean Fever case was infected from an agar culture, which had been previously passed through tap-water and urine.

In this the *M. melitensis* was regularly recovered, growing normally up to 41 days, the urine infected remaining perfectly clear, and was only slightly alkaline when the organism died out.

No. 6.—Faintly alkaline sterilised urine, rich in urates, of a case in the ward was infected by an agar culture derived from a sea-water one, inoculated from an artificially infected urine.

In this the *M. melitensis* again retained vitality for an exceptionally long period, viz., 39 days, the last subculture reacting normally in all respects.

Viability in Sea-Water.

Series 2.—These were made with ordinary sea-water taken from the harbour, sterilised, and, after inoculation, placed in the 22° C. incubator, and evaporation prevented.

No. 1.—Ten c.c. of sterilised sea-water was infected from a five-day old agar culture of *M. melitensis* derived from artificially infected urine. In the subcultures the colonies gradually became fewer, the last being found on the 26th day.

No. 2.—Ten c.c. of sterilised sea-water was infected with 1 c.c. of seven-day old broth culture of *M. melitensis*, which had been derived from artificially infected urine. From this the last successful subculture was made on the 21st day, when the tube was accidentally broken.

No. 3.—Ten c.c. of sterilised sea-water was infected with 1 c.c. of slightly alkaline urine strongly infected with *M. melitensis* derived from artificially infected urine, that is, two passages through urine; subcultures gave abundant growth until 30 days, when it appeared to die out rapidly, the last being obtained on the 34th day.

Viability in Tap-Water.

Series 3.—These were made from sterilised tap-water of the laboratory after inoculation, being kept in 22° C. incubator.

No. 1.—Ten c.c. of sterilised tap-water was infected from an agar

culture of *M. melitensis* derived from artificially infected urine. The last successful subculture was obtained on the 23rd day.

No. 2.—Ten c.c. of sterilised tap-water was infected with 1 c.c. of a seven-day old broth culture of *M. melitensis* derived from artificially infected urine. Growth was obtained up to the 18th day, when the tube became contaminated.

No. 3.—Ten c.c. of sterilised tap-water was inoculated with 1 c.c. of slightly alkaline urine freshly infected with *M. melitensis* which had been grown in urine. Growth was obtained abundantly until the 26th day, the last being obtained on the 30th.

Viability in Fabric which had been Infected, and Dried in Hot Incubator.

Series 4.—Small squares of flannel fabric were used. These were soaked for certain periods in the culture, drained, and then dried slowly in the hot incubator.

No. 1.—The squares of fabric were immersed for 24 hours in a broth culture of *M. melitensis*, then dried as above stated. One of these was removed every three days, placed in broth, and finally subcultured on agar. Recovered for 37 days.

No. 2.—Immersed for a quarter of an hour in infected urine. Recovered for 7 days only.

No. 3.—Immersed for half an hour in grossly infected urine. Recovered for 15 days.

No. 4.—Squares soaked for 24 hours in urine (Series 1, No. 5) on 10th day of growth. *M. melitensis* recovered for 26 days. The squares soaked became discoloured and stiff from impregnation with urinary constituents.

No. 5.—Squares soaked in infected sea-water for 12 hours. No growth obtained on the 7th day.

Viability in Artificially Infected Dust.

Series 5.—Fine bath brick dust was sterilised, and then soaked for one hour in infected media, drained, and dried in hot incubator. Subcultures were regularly made from this with litmus broth, and finally from this agar cultures, and tested for purity.

No. 1.—Fine oolitic dust infected for one hour with five-day old broth culture, derived from artificially infected urine. *M. melitensis* recovered for 25 days.

No. 2.—As above. *M. melitensis* recovered for 26 days.

No. 3.—Oolitic dust infected with seven-day old broth culture derived from infected urine. Growth abundant and typical. Recovered up to 36 days.

No. 4.—Oolitic dust soaked in slightly alkaline sterilised urine,

grossly infected with *M. melitensis*. Growth was recovered for 30 days.

No. 5.—Oolitic dust soaked in urine in which *M. melitensis* was growing feebly. Not recovered on 3rd day, or any date after.

Series 5A.—Road dust with vegetable and other debris collected and thoroughly sterilised, and tested by control cultures. Treated in similar manner to oolitic dust.

No. 1.—Road dust infected by soaking in five-day old broth culture of *M. melitensis* derived from artificially infected urine. Recovered for 44 days.

No. 2.—Road dust soaked in urine strongly infected with *M. melitensis*. Recovered for 16 days.

No. 3.—Road dust soaked in urine in which *M. melitensis* was growing feebly. Not recovered on 3rd day, or any subsequent date.

No. 4.—Road dust infected with urine and broth culture of *M. melitensis* in equal parts. Recovered for 8 days only.

Table of Results of Vitality of the *M. melitensis* outside the Body.

Medium tested.	Source of supply.	Number of days on which the <i>M. melitensis</i> was recovered.
Urine, sterilised.	Agar culture	9
	"	12
	"	18
	"	39
	"	41
Sea-water, sterilised	Broth culture	14
	"	21 (tube broken)
	Agar culture	26
	Infected urine	34
	Infected urine	30
Tap-water, sterilised.	Agar culture	28
	Broth culture	18 (contaminated)
	Infected urine	30
	" $\frac{1}{2}$ hour.	7
	" $\frac{1}{2}$ "	15
Fabric, infected and dried..	" 24 hours.	26
	Broth culture 24 "	37
	Sea-water 12 hours	Less than 7
	Broth culture	25
	"	26
Dust, oolitic, infected and dried	"	36
	Infected urine (strong)	30
	" (weak)	Under 8
	Broth culture	44
	Infected urine (strong) ..	16
Road dust, infected and dried	" (weak)	Under 8
	Urine and broth	8

OBSERVATIONS ON THE VIRULENCE OF *MICROCOCCUS MELITENSIS* FOR THE GUINEA-PIG.

By J. W. H. EYRE, M.D., F.R.S. Edin., Lecturer on Bacteriology in the Guy's Medical and Dental Schools, Bacteriologist to Guy's Hospital, etc.

Summary.

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The Pathogenic Action of M. melitensis.

The degree of virulence possessed by *M. melitensis* for such rodents as the rabbit and guinea-pig is naturally exceedingly low, and in order to produce a fatal infection in these animals, it is necessary to introduce enormous numbers of cocci subcutaneously or intraperitoneally; even then the infection follows a protracted course, and weeks or even months may pass before death takes place.

The majority of the animal experiments by early observers was carried out upon the monkey, an animal which, as originally shown by Bruce,* and afterwards by Hughes, can be easily and certainly infected, chiefly for the reason that attempts to produce fatal infection in the usual laboratory rodents (the rabbit, guinea-pig, and mouse) were for the most part unsuccessful, certainly no appreciable increase in virulence could be demonstrated.

In 1898, however, Durham† applied the method Catani had used to

* Bruce, "Sur une nouvelle forme de fièvre rencontrée sur les bords de la Méditerranée," *Annales de l'Institut Pasteur*, vol. 7, 1893, pp. 289—304; 'Practitioner,' 40, 1888, p. 241; Hughes, "Investigations into the Etiology of Mediterranean Fevers," 'Lancet,' vol. 2, 1892, p. 1265.

† Durham, "Some Observations on the *Micrococcus melitensis*" (of Bruce), 'Journ. of Path. and Bact.,' vol. 5, December, 1898, pp. 377—388.

raise the virulence of the influenza bacillus to *M. melitensis*, and was able to show that the natural resistance of both the rabbit and the guinea-pig was much more readily overcome if the Micrococcus was introduced directly into the brain substance. Further, by a short series of intracerebral passages, he succeeded in slightly raising the virulence, as will be seen from an inspection of the accompanying table, which is compiled from his paper.

Table I.

Animal.	Inoculation.	Dose.	Mode of infection.	Result.
1. Rabbit....	Large quantity of cocci from agar plate suspended in 4 c.c. of a 4-day broth culture	0.5 c.c.	Intracerebrally	Died 4th day.
2. Guinea-pig	—	0.3 „	„	Ill from 3rd to 10th day; recovered. Killed at 4 months.
4. Guinea-pig	5-day agar culture from Rabbit 1	1.5 loop (3 mgrms.)	„	Died 6th day.
5. Guinea-pig	Agar culture from Guinea-pig 4	1 loop (2 mgrms.)	„	Died 4th day.

The rise in virulence for the guinea-pig resulting from one passage through the rabbit is remarkable; but it will be seen that the additional passage through Guinea-pig 4 did not cause any appreciable effect—the fact that a slightly smaller dose was fatal to Guinea-pig 5 might be readily explained on the ground of that animal's greater susceptibility, *e.g.*, a younger or a smaller animal—a contention which is supported by the observations that in subsequent inoculations 2 and 1 loop (*i.e.*, 4 and 2 milligrammes) were fatal respectively in four and six days.

This feeble virulence for the guinea-pig combined with the tardy growth of *M. melitensis* upon artificial media renders it a matter of some difficulty to estimate the amount, or indeed the presence of protective bodies in the serum of animals treated with cultivations of the organism—so troublesome a process in fact, that after attempting, during the early part of last year, to prepare an anti-serum from the goat, I suspended my experiments in favour of an inquiry into the possibility of exalting the virulence of the micrococcus.

In view of the results of Durham's observations, I decided to limit my endeavour to exalt virulence for the guinea-pig to the intra-cerebral method of inoculation, and to employ, at first, one strain only of the *M. melitensis* in my experiments. The result was distinctly encouraging, for after rather more than a score of passages a very considerable access of virulence was obtained.

Before giving the details of these passages, a few points concerning the organism used and the methods followed may be of interest.

Organism Employed.—*M. melitensis* "No. 5."

Origin.—Isolated from the pus during life, and subsequently from the spleen (*post-mortem*) of a fatal case of subdiaphragmatic abscess* occurring in Guy's Hospital during October, 1903.

Morphology and Cultural Characters quite typical.

Initial Virulence for the Guinea-pig.—When first isolated, three entire four-day agar cultivations (*vide infra*) emulsified in 0.4 c.c. sterile saline solution, injected intracerebrally, were required to cause death—the fatal termination ensuing about 25 days after inoculation.

Medium Used for Growth of Virulence Cultures.

Previous experiments of my own, no less than the experience of other observers, show that fluid media are quite unsuited for the growth of, what I may term "Virulence Cultures." In the first place, growth in, for example, nutrient broth is extremely slow, and does not reach its maximum until the 6th to 8th day: then, too, the cranial capacity of the guinea-pig is small, and if the dose of culture exceeds or even amounts to 0.5 c.c., either some of the inoculated fluid at once escapes, or severe pressure symptoms supervene, and the animal dies within a few minutes.

A cultivation upon a solid medium, on the other hand, affords the opportunity of concentrating a large amount of the infective material in a bulk sufficiently small to ensure retention of the entire dose within the cranial cavity, without causing the exhibition of pressure symptoms, by scraping off large numbers of cocci and emulsifying in minute quantities of sterile saline solution.

Some observations upon the cultural characters of *M. melitensis* made* during last summer by the writer in conjunction with Surgeon Duncan, R.N., showed that upon ordinary nutrient agar (prepared and standardised to +10, according to the method and scale I have described elsewhere)† *M. melitensis* in subcultivations would develop colonies

* Eyre, "A Case of Subdiaphragmatic and Hepatic Abscess consecutive to Mediterranean Fever," 'Guy's Hospital Reports,' 59, 1905, pp. 207—216.

† Eyre, "Standardisation of Nutrient Media," 'Brit. Med. Journ.,' 2, 1900, p. 921, and 2, 1901, p. 788.

visible to the naked eye in from 24 to 30 hours at 37° C., and would attain the maximum development in from 72 to 96 hours.*

I therefore decided to employ this medium in the form of "slant" tube cultures, filling the medium in quantities of 10 c.c. into tubes specially selected to secure uniformity of size, and always slanting the medium at about the same angle in order to obtain approximately equivalent areas for growth in each culture.

Preparation of Inoculum.

Tubes were inoculated from the spleen of one guinea-pig which had succumbed to intracerebral infection for inoculation into the brain of the next animal of the series, and after 24 hours' incubation at 37° C. were examined naked eye, and microscopically by means of smear preparations. As a rule at this age no definite colonies could be distinguished naked eye, although the inoculated surface of the medium presented a ground-glass appearance, and film preparations always yielded abundant evidence of growth.

After the preliminary examination, a sterile platinum loop was introduced into the tube, moistened in the water of condensation, and then gently rubbed all over the slanted surface of the medium. As the result of this manoeuvre, by the third or fourth day the medium was covered with a luxuriant growth, affording ample material for inoculation.

Method of Measuring the Dose of Inoculum.

The terms "entire culture," "half a culture," etc., as applied to dosage have little to recommend them on the score of exactitude, therefore the more accurate method of measurement by "loops" evolved by the late Dr. Washbourn and myself for the estimation of the virulence of the pneumococcus was utilised in the experiments. Briefly, this method consists in using a platinum loop accurately calibrated by weighing experiments; filling it carefully with the culture, and then emulsifying its contents in a definite measured quantity of broth, and using for the inoculation portions of this emulsion, representing fractions of the original loop. The special loop I use is the one originally made for pneumococcus work; its holding capacity had already been estimated to equal 0.5 milligramme of a 24-hour-old blood agar cultivation of pneumococcus; and when re-calibrated for 72 to 96-hour-old agar cultures of *M. melitensis* it was determined to have an identical capacity.

* At the same time I am not prepared to state absolutely that +10 is the optimum reaction of the medium to be employed for this purpose, until some experiments that are being carried out now are completed. Still this reaction is undoubtedly close to the optimum, so that in the absence of more definite knowledge I did not feel inclined to deviate from it.

Further, by plating out various fractions of a loopful of 72 to 96-hour-old agar cultivation and enumerating the colonies that subsequently developed, an approximate estimation was obtained of the number of cocci per loopful—that is to say, contained in 0.5 milligramme cultivation.

The average determined from a large series of experiments worked out to 1,250,000,000 cocci per loopful.

Now as in some of the preliminary inoculations more than one "slant" tube culture was required to produce a fatal infection, several observations were made to determine the average number of loopfuls per cultivation. After many trials this was found to be 25; and all the doses in the detailed table of inoculations are calculated as "loops," and so recorded—although in the first few inoculations the figures can only be regarded as approximately correct, for these doses were not measured accurately, on account of their size.

Method of Inoculation.

As the intercranial method of inoculation is not amongst those most commonly practised, some details of the *technique* I adopted may be of interest.

The guinea-pig is first fully anæsthetised by means of a mixture of alcohol, chloroform, and ether, in the proportion of 1 : 2 : 6 (A_1 , C_2 , E_6), administered on a piece of absorbent cotton-wool placed either in the corner of a folded towel, or in the bottom of a small conical glass beaker.

The animal is then fastened down to the operating table, or firmly held by an assistant, and the hair of the scalp moistened with a solution of soft soap in 2-per-cent. lysol, which, with the help of hot water and cotton-wool, is worked up into a lather. The entire scalp, from the occipital protuberance to the root of the snout, is shaved, and finally washed with warm lysol solution.

A median incision commencing over the occiput and running forwards for about 2 cm., is made through the skin and subcutaneous cellular tissue, and retractors, secured by the assistant, used to hold open the wound. The periosteum is next divided along the entire line of the skin incision, then raised with a blunt dissector and also secured by the retractors.

A small nasal trephine (Curtis's), having a tooth-cutting circle of 6 mm. diameter,* is attached to a dental engine, and a small disc of bone removed from the left parietal bone; this trephine hole is cut well to one side of the median line to avoid injuring the superior

* This instrument has been adapted for me by Messrs. Down Bros. by the addition of an adjustable collar guard, secured by a screw, to prevent laceration of the dura mater or brain substance.

longitudinal sinus, a mishap which gives rise to troublesome hæmorrhage.

A hypodermic syringe provided with a fine needle is used to inject the measured dose of cocci, and some little manipulation is found to be necessary to ensure that the animal receives the entire dose. The injection may be made into any portion of the brain substance, or into the subdural space. Usually I inject into the left cerebral hemisphere, rarely into the frontal region. I avoid entering the cerebellum solely because muscular tremors and twitchings of the entire body are thereby induced which last for some minutes and interfere with the suturing of the skin wound.

The disc of bone is replaced or not, according to circumstances. If the injection appears to have caused any appreciable rise in the intracranial pressure, as indicated by protrusion of brain matter and meninges into the trephine circle, I do not replace the bone; otherwise I do. The periosteum is now readjusted as nearly as possible to its original position, and the skin incision closed by means of a continuous suture of either linen or silk, then sealed with flexile collodion. A dressing of sterile absorbent cotton-wool is fixed over the wound with more collodion, and the animal allowed to come round from the anæsthetic. Although the description is lengthy, the operation occupies but little time; given one assistant to attend to animal and the anæsthetic, 10 minutes will suffice from the commencement of the anæsthetisation to the return of the guinea-pig to its cage.

Passages to Exalt Virulence.

For the sake of brevity and clearness, I have tabulated the details of such of my inoculation experiments* as are pertinent to the present inquiry (*vide* Table II), and in this connection I must distinctly point out that my object was in no sense to determine the *minimal* fatal dose of that particular strain of *M. melitensis* I employed, for I take it the minimal fatal dose is the smallest dose which will cause a fatal specific infection after the lapse of no matter how lengthy a period.

Such an inquiry would have required the expenditure of more time than is at the disposal of any one man, for Durham has already shown, and I can fully confirm his observations, that an experimental animal may die of *M. melitensis* infection at a period as far distant as three months from the date of inoculation.

My intention was rather to so raise the virulence of the coccus for the guinea-pig that a comparatively small, and accurately measurable dose, should consistently cause death within a definite period of seven days; and in tabulating my results I have been guided by this principle, and have restricted myself as far as possible to the inclusion of

* Eyre, "The Preparation of Nutrose Agar," 'Trans. Path. Soc.,' 55, 1904, p. 91.

those animals in any given series that succumbed within seven days to the smallest dose. For instance, Guinea-pig 12 was in fact labelled (B) of a series of three inoculations performed on the same day with different doses of the same culture. (A) received 25 loops, and died in 21 hours; (C) received 0.1 loop, and died in 12 days; therefore (B), having received the smallest dose (1 loop) that was fatal within the prescribed period, alone appears in the table.

From the details shown it will be seen that after 21 passages through guinea-pigs the virulence of a particular strain of *M. melitensis* originally so feeble that 75 loops (or 37.5 milligrammes) of culture required 25 days to kill a 380-gramme guinea-pig, has been so exalted that two loops (or 1 milligramme) of culture is sufficient to kill a 590-gramme pig in about 24 hours, whilst 0.5 loop (or 0.25 milligramme) will kill a 350-milligramme pig within five days.

Course of the Infection.

The course of the infection of the guinea-pig by *M. melitensis* may be conveniently considered under two separate headings: (1) Acute, and (2) Chronic, according to whether death is caused in a few hours or days, or is delayed for from one week to two or three months.

Acute Infection.—An animal dying within a few days of intracerebral inoculation with a moderate dose of a highly virulent cultivation or a large dose of a less virulent one, supplies the type for this form of *M. melitensis* infection.

A short incubation period varying in duration from 12 to 24 hours follows the inoculation, and during this time the animal appears to be in normal health and eats well, although the progressive loss of weight which is the marked characteristic of the infection begins within a few hours of inoculation. A stage of irritation follows the incubation period, and lasts for about 24 hours; it is marked by convulsions, at first localised and produced in response to direct stimuli; afterwards becoming generalised, tonic in character and occurring at frequent and irregular intervals; progressive muscular weakness is a marked feature of this stage, throughout which the animal is obviously ill and stupid, and refuses food. The stage of irritation passes gradually into one of coma, with paresis or paralysis, affecting first the hind legs, afterwards involving the fore limbs also. Handling or even touching will at first rouse the animal and provoke general convulsions; later, the guinea-pig falls on its side, becomes insensible, and, in fact, appears moribund. In this condition, however, the animal may remain for 24 or even 36 hours, and during the latter part of this period no rectal temperature can be recorded by the ordinary clinical thermometer, for 32° C. is hardly ever exceeded. Death is sometimes preceded by convulsions, but usually no such warning is given. To give a concrete

illustration of the train of symptoms and *post-mortem* findings in these acute infections I cannot do better than cite in full the clinical history of and autopsy on guinea-pig 19 (*vide* Table II), which is quite typical. Incidentally, I may mention that this case would serve equally well to illustrate the course of infection in the rabbit.

Guinea-pig 19. Sex ♂. Weight 450 grammes. Temp. 38° C.

11.2.05	4 P.M.	A.C.E. was administered, and a 6 mm. trephine circle was cut from left parietal bone. Four (4) loops of 3-day old agar cultivation of <i>M. melitensis</i> from spleen of Guinea-pig 18, emulsified in 0.2 c.c. sterile saline solution, injected into substance of left cerebral hemisphere. Disc of bone replaced, also periosteum, skin incision sutured, and wound dressed with collodion and cotton-wool.
	12 P.M.	Appears quite well. Has eaten well since inoculation.
12.2.05	9 A.M.	Is huddled up in one corner of cage; is not eating; hair dull and standing on end; is obviously ill. Has lost 60 grammes in weight.
13.2.05	„	Condition apparently unchanged. Has lost a further 60 grammes in weight.
	10.15 A.M.	Is now grinding teeth, moves slowly, and, if turned on back, rights itself very slowly.
	10.30 A.M.	Generalised spasms result if touched; convulsive movements occur from time to time even in the absence of obvious stimuli.
	1 P.M.	Much worse—marked paresis of hind quarters.
	2 P.M.	Convulsive "circus" movements occur from time to time—the animal dragging itself round "clockwise" by means of its fore paws.
	9 P.M.	Quiet in corner of cage; breathing laboured.
14.2.05	9 A.M.	Apparently unconscious, lying on side; if placed on legs is unable to stand, and falls down after slight feeble convulsive movements, once more becomes still. Breathing shallow and slow.
	11.30 A.M.	Condition unaltered.
	11.45 A.M.	Still in same position as when last looked at. Is now dead.

Post-mortem Examination.

Scalp Incision.—Scalp wound healthy in appearance, lips of incision healing by primary union; no signs of pus visible in wound; no stitch abscesses.

Subcutaneous tissue occupied by oedematous and jelly-like exudation marked here and there with small hæmorrhages.

Bone.—The disc of bone is firmly fixed *in situ* by serous exudation, in which the periosteum is also involved. On raising the disc of bone no protrusion of meninges, etc., occurs.

Cranial Cavity.—(This is most conveniently reached without damage

to the structures beneath by reflecting skin and periosteum from over the entire cranial vault; then, with a red-hot iron, thoroughly searing the bone, which when seared and dry can easily be broken up with a pair of forceps, and removing it piece-meal, starting from the trephine circle.)

General congestion and injection of the vessels of the dura mater, the site of inoculation being marked out by an area of bloody lymph, roughly corresponding in size and shape to the circle of bone removed from the calvarium. On removing the meninges a thick layer of yellowish lymph is seen adhering to the surface of the convolutions in the left parieto-occipital region (this microscopically consists of a dense mass of large mononuclear leucocytes, permeated throughout by masses of cocci); cerebral vessels greatly engorged and numerous petichial hæmorrhages visible on the surface of the brain.

The cerebro-spinal fluid is more or less increased in amount and contains numerous micrococci, free and also included in cells.

On section there are numerous small hæmorrhages scattered throughout the brain substance, whilst the *velum interpositum* is so congested as to resemble a clot of blood. Elsewhere the brain appears unaffected to the naked eye. Agar tubes inoculated with brain substance from any portion of cerebrum or cerebellum or from cerebro-spinal fluid, either cerebral or spinal, yield a copious and pure growth of *M. melitensis* within 36 hours.

Thoracic Cavity.—Slight enlargement of anterior mediastinal and of bronchial glands. Small quantity of clear serous effusion in the pleural cavity. Cultivations from this fluid remain sterile. Few hæmorrhages on the surface of lungs. Pericardium distended with clear serous fluid, also sterile. Blood removed from right side of heart plated in agar yields, on an average, some 35 colonies per cubic millimetre.

Peritoneal Cavity.—Excess of clear blood-stained fluid in peritoneal cavity, sterile on cultivation. Gall-bladder distended with clear bile. Liver, spleen, kidneys dark and engorged with blood, the spleen being distinctly enlarged.

Cultivations from liver and spleen give good growth of *M. melitensis* within 48 hours. Kidney pulp yields only a few scattered colonies of *M. melitensis*.

Omentum injected; a few enlarged mesenteric glands noted.

Bladder distended with turbid urine. Cultivations prepared from the centrifugalised deposit of the few cubic centimetres of urine contained in the bladder remain sterile.

Cultivations from the bone marrow from practically all the long bones yield a more luxuriant growth of *M. melitensis* than from other organs, with perhaps the exception of the spleen and brain.

Chronic Infection.—As in the present instance we are not particularly concerned with chronic infection, I shall dismiss this subject very briefly.

After intracerebral inoculation with a very minute dose of a highly virulent culture or a fair sized dose of a less pathogenic one the infection pursues an extremely chronic course, and beyond progressive emaciation and profound anæmia presents no very marked or characteristic symptoms. The early symptoms resemble those of the more acute infection above described but are much less severe in character. For instance the incubation period is usually prolonged to two or three days and is followed by a period, extending over from three to six days, during which the animal is distinctly ill and refuses its food, remains huddled up in one corner of its cage, loses weight rapidly and becomes extremely weak. Convulsions of a mild type can usually be provoked at the beginning of this stage by handling the animal or by turning it on to its back.

The animal then gradually recovers, eats well—even ravenously—and although the emaciation may be arrested for a while, the original weight is never entirely recovered. After an interval extending over weeks or even months, during which, except for emaciation, the animal appears in perfect health, death suddenly takes place.

More rarely the animal is obviously ill for two or three days before death, refuses food and becomes comatose just before the end.

Post-mortem Appearances.

Seat of Inoculation.—The site of the skin incision is occupied by a firm linear scar usually adherent to the periosteum and bone beneath, the disc of bone, if it has been replaced, has usually united completely.

Cranial Cavity.—Slight injection of meningeal and cerebral vessels usually present—brain substance appears normal.

Cultures from brain substance and cerebro-spinal fluid yield only a scanty growth of *M. melitensis* or remain sterile.

Thoracic Cavity.—Lungs usually anæmic, otherwise normal; cultures from heart blood remain sterile.

Peritoneal Cavity.—Peritoneum and intestines blanched and anæmic; no subperitoneal, omental or mesenteric fat visible; otherwise viscera normal. Cultivations from liver remain sterile; those from spleen and bone marrow may or may not yield a scanty growth; on the other hand those established from the centrifugalised deposit of the turbid urine or even from the urine itself give rise to fairly good growth.

Mode of Exit of M. melitensis from the Body.

A matter of the highest practical importance, but one upon which I have not yet touched, deals with the route or routes by which *M. melitensis* leaves the animal body. So far as concerns what we have termed “chronic” infections, *M. melitensis* can be readily isolated from catheter specimens of the urine throughout the course of the infection and also from urine taken directly from the bladder *post-*

mortem, even when intervals measured by months have elapsed since the inoculation, and no matter what the path of the original infection of the animal has been—intraperitoneal, subcutaneous, or intracranial. This was shown first by Durham, and his results, so far as concerns the guinea-pig and rabbit, have subsequently been as fully confirmed by the observations of other workers as by my own experiments; Horrocks and Shaw have recorded analogous results, too, from observations upon the human subject.

When, however, we consider the results of observations upon the *acute* infections, we find that *M. melitensis* is not consistently present in the urine. In about 50 per cent. of the animals dying within five days of inoculation, I have failed to detect the Micrococcus in the urine, although I have employed the entire contents of the bladder for the insemination of culture tubes and plates.

During the experiments detailed above, which had for their primary object the exaltation of virulence, I systematically examined many of the organs of the infected animals by cultural tests, using a special circular loop of 1.5 mm. diameter, and carefully compared the amount of the resulting growths.

My observations soon convinced me that the most copious growth per loopful of material was obtained from the brain substance, next in order and all about equal came the liver, spleen, and long bone marrow. Finding such large numbers of cocci present in the liver tissue, I naturally turned my attention to the contents of the gall bladder. In my first observations I seared the surface of the gall bladder with a red hot iron at some convenient spot, punctured the wall with a Pasteur pipette, and aspirated some of the bile in order to prepare my cultivations.

My results were unsatisfactory and inconsistent—sometimes a good growth of *M. melitensis* was obtained, at others the cultures remained sterile. My positive results, I noticed, were obtained when the cultures were made immediately after death; usually, when the *post-mortem* examination was not performed until some hours after death, a negative result was recorded.

It was next observed that if the cadaver was allowed to remain undisturbed, sedimentation occurred in the bile, and the cocci became collected into large flocculent masses deposited near the mouth of the common bile duct, so that if cultivations were made from the supernatant bile no growth resulted, although good growth could be obtained from the before-mentioned flocculent masses when these were taken up by the pipette in aspirating the last portion of bile.

Following the course of the bile, in my subsequent *post-mortem* examinations I prepared plate cultivations (using the modified Drigalaki and Conradi “nutrose” medium that I have already described in connection with some dysentery investigations) from

Table II.—Intracerebral Passages to Exalt Virulence.

Guinea-pig. No. in series.	Sex.	Weight in grammes.	Dose of inoculum.		Bulk of inoculum.	Duration of infection.	Weight at death, in grammes.	Observed loss of weight.	
			In loofula.	In milligrammes.				Total in grammes.	Percentage of body weight.
1	♂	380	75	37.5	0.4	25 days	180	220	78.5
2	♀	420	25	12.5	0.3	15 "	160	270	64.3
3	♂	460	25	12.5	0.2	7 "	250	210	45.6
4	♀	700	25	12.5	0.3	9 "	350	350	60
5	♀	750	25	12.5	0.2	2½ "	630	130	17.3
6	♀	480	25	12.5	0.2	2½ "			
7	♀	560	25	12.5	0.2	2 "			
8	♀	560	12	6	0.3	2 "	450	110	19.6
9	♂	880	12	6	0.3	3 "	710	180	20.2
10	♂	570	12	6	0.2	3½ "	460	110	19.3
11	♂	870	6	3	0.2	2 "	270	100	27
12	♂	310	1	0.5	0.2	4½ "	300	110	35.4
13	♂	240	12	6	0.2	2½ "	210	180	54.1
14	♂	240	15	7.5	0.2	4 "	210	180	54.1
15	♂	240	5	2.5	0.1	24 hours	200	40	16.6
16	♀	240	8	4	0.2	2 days	165	75	31.2
17	♂	180	6	3	0.2	2 "	160	80	16.6
18	♂	850	6	3	0.2	2½ "	330	30	8.5
19	♀	450	4	2	0.2	3 "	280	170	37.7
20	♂	260	♂	*	*	21 hours	210	40	16
21	♀	380	2	1	0.1	3½ days	270	110	28.9
22	♀	590	2	1	0.3	24 hours	460	130	22
23	♀	360	0.5	0.25	0.1	6 days	280	120	34.2
24	♂	460	0.5	0.25	0.1	2 "	360	100	21.7
25	♂	480	0.5	0.25	0.1	3 "	330	110	25.5

* Owing to error, no note was made of the size of the dose.

Table III.—*Post-mortem Findings.*

Guinea-pig. No. in Series.	<i>Micrococcus melitensis</i> recovered in culture from—																
	Brain sub- stance.	Heart blood.	Serous effu- sion in thorax.	Spleen.	Liver.	Bile.	Peritoneal fluid.	Kidney.	Urine.	Long bone marrow.	Duodenum.	Jejunum.	Ilium.	Cæcum.	Rectum.	Fæces.	Intestinal mucus.
1	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
2	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
3	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
4	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
5	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
6	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
7	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
8	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
9	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
10	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
11	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
12	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
13	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
14	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
15	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
16	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
17	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
18	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
19	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
20	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
21	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
22	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
23	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
24	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+
25	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+

+ = growth of *M. melitensis*. ± = scanty growth of *M. melitensis*. - = no growth of *M. melitensis*. 0 = not examined.

scrapings from the walls of the bile duct, and from the mucous membrane lining of the duodenum, and had no difficulty in demonstrating the presence of fair numbers of the Micrococci in both these situations. Proceeding onwards along the intestinal canal, positive results were obtained from the upper portions of the jejunum, but owing to the enormous numbers of purely intestinal bacteria which rapidly overgrew the plates, I was at first unable to demonstrate *M. melitensis* in the lower portion of the jejunum, the ileum, cæcum or rectum.

With the death, however, of Guinea-pig 20 (*vide* Table II) within 24 hours of inoculation, a positive result was obtained from each of these situations, although as the rectum was empty no observations could be carried out with regard to the characteristic faecal masses.

Guinea-pig 21 (*vide* Table II) was deposited in a sterilised glass dish whilst in a moribund condition, so that when the contents of the rectum were expelled at death an hour later, it became a simple matter to prepare plate cultivations from intestinal mucus and faeces separately.

From the mucus a fair number of Micrococci was isolated; the faecal masses gave considerably more trouble on account of the numerous members of the coli and streptococcus groups that were present, but eventually several colonies were isolated and completely identified with *M. melitensis*.

As will be seen from the details of the *post-mortem* examinations (Table III), these results were confirmed more than once.

From the results of these experiments, therefore, the assumption is justified that *M. melitensis* leaves the body of experimental animals by way of the intestinal tract, and possibly by way of the urinary tract also, when the infection is of the acute type. That the coccus leaves the body by way of the urinary tract *alone* when the infection is of a more chronic character appears probable also, for not only have I not succeeded in isolating it from the alimentary canal or faeces of such animals, but cultivations from liver and bile have always yielded negative results.

Conclusions.

1. By a series of intracerebral inoculations, comprising rapid passages from guinea-pig to guinea-pig, the virulence of *M. melitensis* can be exalted to a high pitch for this particular animal.

2. The virulence finally obtained in the present series is such that a small and accurately measurable dose of cultivation corresponding to about 0.25 milligramme in weight, or to rather more than 6,000,000 cocci, will consistently cause death in about five days.

3. Experimental observations show that in these acute infections *M. melitensis* leaves the body of the inoculated animal by way of the alimentary canal, in the intestinal mucus and the faeces, as well as occasionally by way of the urinary tract (in the urine).

ROYAL SOCIETY.

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REPORTS
OF THE
COMMISSION FOR THE INVESTIGATION
OF MEDITERRANEAN FEVER.

Part I.

Containing Reports by

Major HORROCKS, R.A.M.C., Staff-Surgeons GILMOUR and
SHAW, R.N., and Dr. ZAMMIT.

REPORTS
OF THE
COMMISSION
APPOINTED BY
THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,
FOR THE INVESTIGATION OF
MEDITERRANEAN FEVER,
UNDER THE SUPERVISION OF AN
ADVISORY COMMITTEE
OF
THE ROYAL SOCIETY.

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INTRODUCTION.

In the Introduction to Part I, the history of this investigation into the causation and prevention of Mediterranean Fever was given from its commencement until the end of the summer of 1904.

Major W. H. Horrocks, R.A.M.C., and Dr. R. W. Johnstone, Local Government Board, left Malta at the end of September and arrived in England on October 8, 1904.

The results of the work of the Commission during the summer of 1904 are published in Parts I and II.

At a meeting of the Sub-Committee, held on November 17, 1904, it was decided that Staff-Surgeon E. A. Shaw, R.N., Dr. T. Zammit, Board of Health, Malta, and Captain J. Crawford Kennedy, R.A.M.C., should continue the work during the coming winter, and that Major Horrocks and Dr. Johnstone be asked to return to Malta at the beginning of the following fever season. As Captain Kennedy had been struck off all military duty and was devoting his whole time to the work of the investigation, he was made a member of the Commission.

Major Horrocks returned to Malta about the end of May, 1905, but Dr. Johnstone was unable to take up the work again this summer. Lieut.-Colonel A. M. Davies, R.A.M.C., was therefore appointed a member of the Commission in his place and arrived in Malta on May 28, 1905.

Colonel Bruce, C.B., F.R.S., R.A.M.C., the Chairman of the Sub-Committee, left England on May 19, 1905, and proceeded to Malta, where he met the members of the Commission. Staff-Surgeon Shaw and Captain Kennedy handed to him the papers which form part of the present volume. Dr. Zammit informed him that on account of the pressure of other duties he had been able to do but little work for the Commission, but that he would now be able to devote his whole time to it. He communicated some notes on the feeding of goats with *Micrococcus melitensis*, which seemed to show that the goat is to some extent susceptible to Mediterranean Fever. The following experiments were made by Dr. Zammit:—

Experiment 1.—White Goat.

To note the effect of feeding goats on material containing *Micrococcus melitensis*.

1904—

- Sept. 15. Examined blood for agglutination. Negative.
- „ 18. Fed this goat, adding the contents of a culture of *M. melitensis* on agar to its food.
- Dec. 3. Blood has reacted in dilutions of 1 in 20 to 1 in 100, but the temperature curve shows no rise. Fed again in the same way.
- „ 23. Blood reacts 1 in 300.

1905—

Apr. 29. Blood reacts 1 in 100. Goat still alive.

Experiment 2.—Red Goat.

1904—

Dec. 3. No blood reaction. Fed one tube agar.

„ 5. Fed again.

„ 15. No blood reaction.

„ 23. Blood reacts 1 in 20 ; 1 in 50 after half-an-hour.

1905—

Apr. 29. Blood reacts 1 in 50.

Dr. Zammit informed the Chairman that he considered goats to be susceptible to Mediterranean Fever, and that the disease is spread to human beings by goats. A temporary laboratory was set up in the Lazaretto buildings, Fort Manoel, to continue the investigation of the disease in goats and also the transference of the disease by mosquitoes.

Colonel Bruce returned to England on June 12, 1905.

On June 23, 1905, Major Horrocks wrote that he had discovered the *M. melitensis* in the milk of an apparently healthy goat, and on the 26th he further wrote that he had already found the *M. melitensis* in the milk of five goats taken from two different herds, and that Dr. Zammit had found it in the blood of one of these goats. Horrocks also said that the milk of the goat fed by Dr. Zammit last September was still crammed with *M. melitensis*. It would therefore appear that the Commission are on the eve of an important and may be far-reaching discovery.

On July 18, 1905, the Chairman received preliminary notes from Major Horrocks, Captain Kennedy, and Dr. Zammit, on the propagation of Malta Fever by means of goats. These are added to the present volume.

These notes show (1) that one or more apparently healthy goats in every herd are excreting *M. melitensis* in their milk and urine ; (2) that about 50 per cent. of the goats in Malta react to Mediterranean Fever when examined by the serum agglutination test.

It may be objected that no exact proof exists that the drinking of milk containing *M. melitensis* will give rise to the disease in man. When we take, however, into consideration the results of the feeding and inoculation experiments on monkeys, it may be assumed with safety that the disease is propagated in this way, and that no time should be lost in removing such a grave and insidious danger to the public health.

I. ON A QUANTITATIVE BACTERIOLOGICAL EXAMINATION OF THE BLOOD OF 103 MEDITERRANEAN FEVER PATIENTS.

By Staff-Surgeon E. A. SHAW, R.N., Member Mediterranean Fever Commission, Malta.

Blood.

In my September Report I gave the results of the examination of the peripheral blood of 51 Malta Fever patients for the *Micrococcus melitensis* (hereafter referred to as *M. melitensis*). I now give briefly the results of a further series of 52 such cases similarly examined, making a total of 103 cases examined. If any points seem inadequately explained a reference to the first Report will elucidate matters.

Method.—Bend of elbow prepared as for a surgical operation, carbolic acid being the disinfectant used, a pad of lint soaked in 1 in 20 of this being kept on site of intended puncture till the latter was made. Five c.c. of blood drawn off from median basilic vein in graduated sterile serum syringe; 3 c.c. of this placed in a flask containing 60 c.c. of peptone broth, 1 c.c. into a tube containing 19 c.c. of peptone broth and 1 c.c. into a second tube also containing 19 c.c. of broth, and all these well shaken. The flask containing 3 c.c. of blood and one of the tubes containing 1 c.c. of blood were incubated intact. The second tube containing a mixture of 19 c.c. of broth and 1 c.c. of blood was treated as follows: half of its contents were removed with a 10 c.c. sterile pipette and it was then put aside, now containing $\frac{1}{2}$ c.c. of blood and $9\frac{1}{2}$ c.c. of broth, to be incubated; the contents of the 10 c.c. pipette were then added to a 10 c.c. broth-tube, which was well shaken, and now contained 20 c.c. of fluid, $\frac{1}{2}$ c.c. of which was blood; 10 c.c. of this mixture was removed with the 10 c.c. pipette, leaving it containing $\frac{1}{4}$ c.c. of blood, and it was put aside to be incubated; the contents of the pipette were added to another 10 c.c. broth-tube, which in its turn was left with $\frac{1}{8}$ c.c. of blood, and so on, halving the quantity of blood each time till the following series was obtained: flask containing 3 c.c. of blood, tubes containing 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$, and $\frac{1}{512}$ c.c. blood duly numbered and dated, these were then placed in the incubator at 37°, being taken out daily and well shaken to facilitate distribution of the possible *M. melitensis* throughout the medium. At the end of a week glucose-litmus-agar slopes were inoculated by means of a loop from

the 3 c.c. flask, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$ c.c. tubes, these duly labelled and all returned to the incubator. At the expiration of another two days these slopes were examined and those which exhibited growth were put aside to be put through the usual tests for *M. melitensis* (see September Report), and the others with the original broth-tubes returned to the incubator. At the end of a second period of two days the agar slopes were again examined, those showing growth being dealt with as before, and now, it being 11 days since commencement of incubation, those slopes which showed no growth were reinoculated plentifully from the corresponding broth-tubes, and the remainder of the series, the $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$, and $\frac{1}{512}$ c.c. blood broth-tubes, were sub-cultured by means of a large platinum loop on to agar slopes, and all returned to the incubator for a further period of three days, after which all slopes were examined for growth, results recorded, and the examination of that particular specimen of blood terminated.

The foregoing was the general method adopted. It was only slightly departed from in the last 25 cases; in which in order to determine the earliest date at which in these examinations *M. melitensis* made its appearance in the broth, daily sub-cultures were made from the flask containing the 3 c.c. of blood starting from the first day of incubation. As this was never found to be later than the eighth day, and as it will be observed from the foregoing that a total of eleven days' incubation in broth, the same period as observed in the first series of 51 cases, was completed before a blood broth-tube was abandoned as unfruitful, there is obviously an ample margin of safety, and I do not consider that there was any possibility of any fruitful blood broth-tube having been overlooked.

The following table gives the result in a very compressed form; it seems to me unnecessary to write out each blood examination separately. In the remarks which follow the tables, such cases as call for it are discussed in greater detail individually. As all the cases in this series were English and male, nine patients belonging to the navy, and 43 to the army, no column descriptive of nationality and sex has been necessary. In the column "stage of fever," the word "wave" refers to waves of raised temperature. In the column giving patients' temperature for a few days prior to bleeding, the last temperatures are those for the morning and evening of the day blood was drawn, those preceding it being arranged in regular chronological order; the intention being by comparing these, in the cases yielding *M. melitensis*, to ascertain if any relation existed between course of temperature and presence of *M. melitensis* in the blood; these temperatures are given in the form of a fraction, the numerator being the morning and the denominator the evening temperature, the one taken about 8 A.M. the other about 5 P.M. Day of disease, as before, has been calculated from the day the patient first began to feel ill, not from date of

admission into hospital. In each case the highest dilution of the patient's blood serum (determined from a portion of the blood taken for bacteriological examination), which gave a distinct agglutination reaction in a quarter of an hour under the $\frac{2}{3}$ -in. objective of the microscope, has been worked out and is given in the appropriate column as a dilution of 1 in ..., the unit being serum, the other numeral being so many equivalent bulks of "normal" physiological salt solution.

In the last column "recovery of and smallest quantity of blood yielding *M. melitensis*," "Nil" signifies that in this case the result of the examination was negative. The minimal quantity of blood yielding *M. melitensis* is given as in decimals of a cubic centimetre, a preference having been expressed for this mode of presenting it. To facilitate comparison with the first series in which this quantity was expressed as a fraction, I give the equivalent from $\frac{1}{8}$ onwards—

$$\begin{array}{lll} \frac{1}{8} \text{ c.c.} = 0.1250 \text{ c.c.} & \frac{1}{16} \text{ c.c.} = 0.0625 \text{ c.c.} & \frac{1}{32} \text{ c.c.} = 0.0312 \text{ c.c.} \\ \frac{1}{64} \text{ c.c.} = 0.0156 \text{ c.c.} & & \frac{1}{128} \text{ c.c.} = 0.0078 \text{ c.c.} \\ \frac{1}{256} \text{ c.c.} = 0.0039 \text{ c.c.} & & \frac{1}{512} \text{ c.c.} = 0.0019 \text{ c.c.} \end{array}$$

In the chronological table which comes immediately after the foregoing, fractions have had to be resorted to, because when, as in the 17th and 22nd days of the disease, the results of as many as six blood examinations had to be put down in one space, the use of decimals was found to result in an agglomeration of figures in which "definition" was greatly lacking. Here also the foregoing equivalents may be found useful.

63	22	In 1st wave	$\frac{98.4}{100}$, $\frac{98.2}{99.2}$, $\frac{98.4}{98.3}$, $\frac{98.6}{98.6}$, $\frac{98.2}{99}$	40th	11.0 A.M., 98° 4	1 in 500	1.00
64	20	{ 1st wave not yet ended	$\frac{100}{102}$, $\frac{97.8}{101}$, $\frac{98.4}{102}$, $\frac{97.8}{100.6}$, $\frac{98.4}{101}$	108th	11.10 A.M., 100°	1 in 40	Nil
65	20	In 1st wave	$\frac{101.6}{104.2}$, $\frac{103}{104}$, $\frac{100.2}{103.6}$, $\frac{98.2}{100.4}$, $\frac{98.4}{100.6}$	16th	11.10 A.M., 100° 2	1 in 40	Nil
66	28	Still in 1st wave ...	$\frac{101.4}{102.8}$, $\frac{100.4}{104}$, $\frac{101.4}{103.6}$, $\frac{101.6}{104.2}$, $\frac{103}{103}$	65th	11.15 A.M., 102°	1 in 500	0.0078
67	20	In 1st wave	$\frac{98.4}{100.4}$, $\frac{99.4}{100.6}$, $\frac{99}{100}$, $\frac{98.8}{98}$, $\frac{98}{100.2}$	22nd	11.10 A.M., 99°	1 in 1000	0.125
68	28	{ Had 2 waves in 1st attack, then out of hospital 31 days, now in 1st wave of relapse	$\frac{98}{100.8}$, $\frac{98.6}{100.2}$, $\frac{99.4}{100}$, $\frac{100.6}{102}$, $\frac{98}{101.8}$	116th	11.25 A.M., 98° 6	1 in 500	Nil
69	23	In 1st wave	$\frac{100.6}{104}$, $\frac{101.2}{103.4}$, $\frac{99.6}{102}$, $\frac{98.2}{99.8}$, $\frac{98.2}{100}$	27th	11.15 A.M., 99°	1 in 20	Nil
70	41	{ In 1st wave of a relapse	$\frac{98.6}{99}$, $\frac{98.2}{99}$, $\frac{98.2}{99.6}$, $\frac{98.6}{100}$, $\frac{98.6}{100.2}$	158th	11.30 A.M., 99°	1 in 40	0.25
71	20	Still in 1st wave ...	$\frac{102}{104}$, $\frac{101.2}{103}$, $\frac{102}{103.4}$, $\frac{101.6}{103.6}$, $\frac{101.2}{102.6}$	56th	12.15 A.M., 102°	1 in 500	0.5
72	21	In 1st wave	$\frac{98.4}{100.8}$, $\frac{99.4}{101.6}$, $\frac{100.2}{101}$, $\frac{99.2}{100.4}$, $\frac{99.2}{100.6}$	17th	11.20 A.M., 98° 8	1 in 1500	0.0625
73	27	In 1st wave	$\frac{99.6}{101.6}$, $\frac{99.6}{103.4}$, $\frac{100.4}{102.2}$, $\frac{101}{103.8}$, $\frac{100.4}{103.6}$	23rd	11.30 A.M., 101°	1 in 2000	0.5
74	25	In 1st wave	$\frac{101.6}{103.2}$, $\frac{103.4}{103.6}$, $\frac{102.2}{103.4}$, $\frac{103.6}{103.4}$, $\frac{101.2}{103}$	18th	11.45 A.M., 102° 1	1 in 2000	0.5
75	24	In 3rd wave	$\frac{98.6}{100}$, $\frac{98.4}{100.2}$, $\frac{98.6}{100.2}$, $\frac{98.2}{99.8}$, $\frac{100}{103.3}$	149th	4.45 P.M., 103° 3	1 in 1300	Nil
76	20	In 3rd wave	$\frac{98.4}{101.2}$, $\frac{98.8}{100}$, $\frac{98.4}{101.2}$, $\frac{99.2}{100.6}$, $\frac{98.5}{102.5}$	120th	5.0 P.M., 102° 5	1 in 800	0.0625
77	21	In 3rd wave	$\frac{98.4}{100.8}$, $\frac{98}{102.4}$, $\frac{98.6}{102}$, $\frac{98.4}{101}$, $\frac{98.4}{99}$	151st	5.10 P.M., 99°	1 in 300	1.00

No. of case.	Age.	Stage of the fever.	Temperature of patient for few days preceding bleeding.	Day of disease.	Time of bleeding and patient's temperature.	Maximum dilution of patient's blood giving agglutination.	Recovery of and smallest quantity of blood yielding <i>M. melitensis</i> .
78	29	In 1st wave	98·4, 98·4, 98, 98·4, 99·4, 102·2, 102·6, 101·6, 103·4, 103·6	35th	10.45 A.M., 100°	1 in 2800	c.c. 1·00
79	20	In 1st wave	98·2, 97·8, 98·4, 98, 97·6	16th	11.0 A.M., 98°·4	1 in 3000	0·125
80	23	In 1st wave	99·6, 101, 100·6, 100, 100·4	17th	11.10 A.M., 98°	1 in 2500	0·5
81	24	In 1st wave	98·4, 98·4, 98, 97·4, 97·4, 97·6, 97·4, 98, 98·4, 99	12th	4.40 P.M., 100°·2	1 in 500	Nil
82	27	In 2nd wave.....	100·2, 98·4, 99, 99, 99, 101, 102·4, 99·2, 100, 100·2, 99, 99·6, 98·4, 99·8, 99·2	67th	4.55 P.M., 101°·6	1 in 1500	1·00
83	27	In 1st wave	102, 101, 101, 102·2, 101·6	18th	5.10 P.M., 100°	1 in 3000	0·5
84	22	In 1st wave	103·4, 101·6, 100·6, 100·4, 100, 98·2, 98·4, 98·4, 98·4, 98·4	16th	5.0 P.M., 101°·5	1 in 2000	5·0
85	21	In 4th wave	99·4, 100·4, 101, 101·6, 101·5, 99, 99·4, 99·4, 99, 98·6, 101, 101·2, 100, 101, 102	118th	5.10 P.M., 102°	1 in 1500	0·25
86	26	In 1st wave of a relapse after normal T interval of 6 weeks	99·2, 99·4, 99·4, 99·6, 100·4, 103, 101·4, 101, 102·4, 103·2	153rd	5.20 P.M., 103°·2	1 in 1600	3·00
87	23	In 1st wave	N, 97, N, 100·2, N, N, N, 101, N, 97·4	12th	10.45 A.M., normal	1 in 200	Nil

88	21	In 1st wave	101 101'4 100'4 100'2 100'2	23rd	11.5 A.M., 100°4	1 in 500	0·0812
89	32	In 1st wave	103 100'6 101 100'4 101'6	21st	11.15 A.M., 100°8	1 in 40	Nil
90	19	In 2nd wave	101'8 101'6 102'2 101'2 101'6	43rd	4.45 P.M., 100°2	1 in 3000	5·00
91	42	In 2nd wave	98'8 98'2 98'2 98'6 98'4	63rd	5.0 P.M., 99°8	1 in 500	Nil
92	22	In 1st wave	103'4 101'6 102 101'2 100'2	41st	5.15 P.M., 99°8	1 in 1200	0·0156
93	20	In 1st wave	99'4 98'2 99'4 98 98'4	21st	4.0 P.M., 103°	1 in 2000	1·00
94	29	In 1st wave	100'6 100'6 102 99'6 99'8	31st	4.15 P.M., 102°8	1 in 2500	1·00
95	35	In 1st wave	99 100 99'8 100 100'6	21st	4.30 P.M., 100°4	1 in 3000	0·0625
96	36	In 1st wave	101'4 101 101'4 101'2 99'8	19th	4.45 P.M., 103°	1 in 2500	0·0812
97	24	In 1st wave	100'2 99'4 101 101'6 101	26th	4.0 P.M., 97°8	p	Nil
98	31	In 1st wave	101'8 103 102'6 101'4 103	21st	4.15 P.M., 103°2	1 in 2500	0·0812
99	19	In 4th wave	102'8 103 102'4 101'6 102'8	66th	4.30 P.M., 100°2	1 in 1000	0·0812
100	25	In 1st wave	100 100'4 99 99'2 97'2	25th	4.45 P.M., 103°5	1 in 1500	0·5
101	25	In 1st wave	98 99'4 98'6 98'6 98'6	32nd	4.15 P.M., 102°	1 in 2400	0·25
102	30	In 1st wave	100'2 101'8 101'8 100'2 101'2	22nd	4.30 P.M., 100°	1 in 2000	0·0625
103	26	In 1st wave	99'6 99'2 99'4 99'2 100	17th	4.45 P.M., 103°	1 in 2200	1·00

Examination of Bloods.

Table showing in chronological order the date of the disease in each of the 103 cases in which blood was taken for bacteriological examination and the result. The word "Nil" means no *M. melitensis* was recovered; the days of disease not represented by a blood examination are shown blank. It will be seen that while many days are blank, others are represented by two to six blood examinations. As stated before, this has been unavoidable; the number of cases willing to submit to venous puncture was too small to admit of selection. The minimal amounts of blood yielding *M. melitensis* in each case are expressed in fractions of a cubic centimetre.

Day of disease.	Recovery and quantity or no recovery.	Day of disease.	Recovery and quantity or no recovery.	Day of disease.	Recovery and quantity or no recovery.
1		37	$\frac{1}{2}$ c.c.	75	
2		38		76	
3		39		77	
4		40	1 c.c.	78	
5		41	Nil, $\frac{1}{16}$, $\frac{3}{16}$ c.c.	79	
6		42	$\frac{1}{16}$ c.c.	80	
7	$\frac{1}{16}$, $\frac{1}{16}$ c.c.	43	5 c.c.	81	
8	$\frac{1}{16}$ c.c.	44		82	
9	Nil, $\frac{1}{16}$, $\frac{1}{16}$, Nil.	45		83	
10	$\frac{1}{16}$, 1 c.c.	46		84	
11	$\frac{1}{16}$ c.c.	47		85	
12	1, Nil, Nil.	48	Nil.	86	
13	Nil.	49	$\frac{1}{16}$ c.c.	87	
14		50		88	
15	Nil, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{16}$ c.c.	51	2 c.c.	89	
16	Nil, 5 c.c.	52		90	$\frac{1}{16}$ c.c.
17	Nil, Nil, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{16}$, 1 c.c.	53		91	
18	Nil, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{16}$ c.c.	54		92	
19	$\frac{1}{16}$, $\frac{1}{16}$ c.c.	55	$\frac{1}{16}$, $\frac{1}{16}$ c.c.	93	
20		56	$\frac{1}{16}$, $\frac{1}{16}$ c.c.	94	
21	Nil, 1, $\frac{1}{16}$, $\frac{1}{16}$ c.c.	57	Nil.	95	$\frac{1}{16}$ c.c.
22	Nil, Nil, 1, $\frac{1}{16}$, $\frac{1}{16}$, $\frac{1}{16}$ c.c.	58		96	$\frac{1}{16}$ c.c.
23	Nil, $\frac{1}{16}$ c.c.	59		97	
24	Nil, $\frac{1}{16}$ c.c.	60		98	$\frac{1}{16}$ c.c.
25	$\frac{1}{16}$, $\frac{1}{16}$ c.c.	61		101	Nil.
26	$\frac{1}{16}$, Nil.	62	Nil.	108	Nil, Nil.
27	Nil.	63		109	
28	Nil, Nil.	64		110	
29		65	$\frac{1}{16}$ c.c.	116	Nil, Nil.
30	Nil, $\frac{1}{16}$ c.c.	66	$\frac{1}{16}$ c.c.	118	$\frac{1}{16}$ c.c.
31	Nil, $\frac{1}{16}$, 1 c.c.	67	1 c.c.	120	$\frac{1}{16}$ c.c.
32	Nil, $\frac{1}{16}$ c.c.	68		149	Nil.
33	$\frac{1}{16}$ c.c.	69	Nil.	151	1 c.c.
34	$\frac{1}{16}$ c.c.	70	$\frac{1}{16}$ c.c.	153	3 c.c.
35	$\frac{1}{16}$, 1 c.c.	71		158	$\frac{1}{16}$ c.c.
36	1, $\frac{1}{16}$ c.c.	72		240	Nil.
		73			
		74	Nil.		

Remarks.—It will be seen from an inspection of the foregoing tables that at any period of this fever up to the commencement of the 6th month of it (158th day) the causal micro-organism may be found in the blood, and in as small a quantity of blood in the course of the 3rd month as in the 1st month.

The smallest quantity of blood from which in this series of 103 observations it has been isolated has been $\frac{1}{256}$ c.c., practically 4 cub. mm., and that only in two cases.

It is unsafe to assume, as one investigator has done, that in any given case the smallest quantity of blood yielding *M. melitensis* contained only one micrococcus. It would be inexact to express the fact that $\frac{1}{256}$ of a c.c. of blood is the smallest quantity of blood yielding *M. melitensis* in a particular case, in the form that this blood contained 256 micrococci per c.c., and equally inexact to state the fact that 0.1 c.c. of blood incubated in 10 c.c. of broth yielded 31 colonies of *M. melitensis* on an agar slope, in the form that this blood contained 310 micrococci per c.c.; or that 0.25 c.c. of blood spread on the surface of an agar in a Petri dish and incubated, yielded 30 colonies of *M. melitensis* as 120 micrococci per c.c. We do not know whether this micrococcus in the blood is free in the plasma, is phagocyted inside a white blood corpuscle, or is present in both these conditions. If inside a leucocyte, we have yet to learn in what period of time the leucocyte can destroy the vitality of the micrococcus. In various experiments made to ascertain the phagocytic power of fresh normal blood on *M. melitensis*, I have frequently seen as many as 20 and 30 micrococci inside one leucocyte, and it is highly improbable that in combining blood with a nutrient medium, the leucocytes are completely fragmented, and any micrococci they may contain evenly distributed through the medium. For these reasons the somewhat cumbrous method of expressing results as minimal quantity of blood yielding *M. melitensis* has been adhered to as having at least the merit of accuracy.

Minimal Quantity of Blood Yielding M. melitensis.

It will be noticed that the minimal quantity of blood necessary to yield *M. melitensis* in these cases varies within very wide limits, from $\frac{1}{128}$ c.c. (Cases 43 and 47) to 5 c.c. (Cases 84 and 90), that is from approximately 4 cub. mm. to 5000 cub. mm. This surely was to be expected; why should it be constant in a series of patients any more than their agglutinating power on *M. melitensis*, which varies from nil to $\frac{1}{1500}$ or $\frac{1}{2000}$; or, indeed, any other clinical phenomenon?

Here seems the most appropriate place to discuss a feature I made mention of in my former report under the name of "skipping"; where *M. melitensis* was found in some of the higher blood dilutions, and absent from some of the lower, these having been "skipped" or "jumped." This occurred in two of the first series of 51 cases, and in

five of the second series of 52 cases. In Case 59 it was recovered only from the broth-tube containing $\frac{1}{16}$ c.c. of blood, all the other tubes remained sterile; excepting the flask containing 3 c.c. of blood here as a total of 2 c.c. of blood was incubated, obviously this had to be reported as a recovery of *M. melitensis* from 2 c.c. In Case 63 *M. melitensis* was recovered only from the 1 c.c. and the $\frac{1}{16}$ c.c. tubes, the others remaining sterile; it is reported as a recovery from 1 c.c. In Case 77 *M. melitensis* was found only in 3 c.c., 1 c.c., and $\frac{1}{16}$ c.c. tubes; it is reported as a recovery from 1 c.c. In Case 84, *M. melitensis* was found only in the $\frac{1}{4}$ c.c. tube, the 3 c.c., 1 c.c., $\frac{1}{2}$ and $\frac{1}{8}$ to $\frac{1}{16}$ c.c. tubes remaining sterile; it is reported as a recovery from 5 c.c. In Case 90, *M. melitensis* was found only in the $\frac{1}{4}$ c.c. tube, the others, 3 c.c. to $\frac{1}{16}$ c.c., remaining sterile; it is reported as a recovery also from 5 c.c.

This phenomenon must, I think, be interpreted as resulting from the small quantity of *M. melitensis* in the circulating blood; in these cases it would seem there was not enough to supply the tubes found sterile, and it would be a matter of chance into which tube the small apparently indivisible amount of *M. melitensis* got.

In no case has the minimal quantity of blood experimented with, $\frac{1}{16}$ c.c. (about 2 cub. mm.) ever yielded *M. melitensis*; in only two cases out of the 103 has the minimal quantity of blood been so small as $\frac{1}{16}$ c.c. (4 cub. mm.). This has a most important bearing on the question of the possibility of the transmission of infection by biting insects such as mosquitoes, which is still *sub judice*.

It is a larger quantity of blood than any biting insect to be found in Malta can contain. Again, with the possible exception of plague, no known bacterial disease has yet been proved to be thus conveyed; this mode of conveyance of infection would appear to be confined to protozoal diseases, and Schandinn's recent work on blood parasites,* in which he demonstrates that in the gnat the "indifferent" spirochaetes are so small as only to be visible when a number have agglomerated, and that they can pass through a Chamberland filter, tends to place yellow fever, hitherto considered doubtful in this regard, amongst the protozoal diseases.

Is there any Relation between Temperature of the Patient and the Presence of M. melitensis in his Blood?

Taking first the temperatures for the few days preceding and including the date of the abstraction of blood, and grouping these in a tabular form together with the results of the blood examinations, we get the following table:—

* "Generations und Wirtwechsel bei Trypanosoma und Spirochaete," 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' Band 20, Heft 3, 1904.

Course of patient's temperature.	No. of cases.	No recovery of <i>M. melitensis</i> .	Recovery of <i>M. melitensis</i> .	Minimal quantities of blood yielding <i>M. melitensis</i> where recovered.
				c.c.
Steady between— 98° and 101°	20	7	13	1, 1 ² , 1, 1, 1, 1, 1 ² , 1 ² , 1 ² , 1 ² , 1 ² , 1 ²
99° and 102°	22	8	14	1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1 ² , 1 ² , 1 ²
100° and 103°	20	4	16	3, 2, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1 ² , 1 ² , 1 ²
101° and 104°	12	3	9	1, 1, 1, 1 ² , 1 ² , 1 ² , 1 ² , 1 ² , 1 ²
102° and 105°	1	—	1	1 ²
Ascending	7	1	6	5, 1, 1, 1, 1, 1 ²
Descending	14	6	8	5, 1, 1 ² , 1, 1, 1, 1, 1 ²
Steady about normal	7	5	2	1, 1
Total cases ...	103	34	69	

Next grouping the cases according to the patient's temperature at the time blood was abstracted, we get:—

Patient's temperature.	No. of cases.	No recovery of <i>M. melitensis</i> .	Recovery of <i>M. melitensis</i> .	Minimal quantities of blood yielding <i>M. melitensis</i> where recovered.
				c.c.
97° to 97°-9... 1	1	1		
98° to 98°-9... 20	20	10	10	1, 1 ² , 1 ² , 1, 1, 1, 1, 1, 1 ²
99° to 99°-9... 21	21	9	12	1, 1, 1, 1, 1, 1, 1 ² , 1 ² , 1 ² , 1 ²
100° to 100°-9... 22	22	7	15	5, 2, 1, 1, 1, 1, 1, 1 ² , 1 ² , 1 ² , 1 ² , 1 ² , 1 ²
101° to 101°-9... 13	13	5	8	5, 1, 1, 1, 1, 1, 1 ²
102° to 102°-9... 15	15	1	14	1, 1, 1, 1, 1, 1, 1, 1, 1 ² , 1 ² , 1 ²
103° to 103°-9... 11	11	1	10	5, 3, 1, 1, 1, 1, 1, 1 ²
Total cases ...	103	34	69	

It will be seen that both these tables show a distinct relation between the presence of *M. melitensis* in the peripheral blood and the patient's temperature; the first showing an increasing ratio of recoveries of *M. melitensis* with the higher temperatures; the second similarly; but no such relationship is visible between temperature and minimal quantity of blood containing *M. melitensis*.

Is there any Relation between the Agglutinating Power of Blood of Mediterranean Fever Cases and the Presence of M. melitensis therein?

When commencing the summer examination of these bloods in June, 1904, to corroborate the diagnosis of Mediterranean Fever arrived at by the medical officer in charge of the case, I invariably examined some of the blood drawn for agglutination reaction on *M. melitensis*, and the diagnosis of the cases selected and given is as certain as it can be. After doing a few cases, it was felt it would be of interest to ascertain what, if any, relation existed between high or low agglutinative power and the presence of *M. melitensis* in the blood. It was accordingly necessary to fix on an arbitrary standard to which all recorded agglutination reactions in the series should conform, in no matter what dilution of serum. For the purpose of this work it was therefore laid down that no agglutination reaction would be recorded unless visible under the $\frac{2}{3}$ -in. objective of the microscope 15 minutes after contact between diluted serum and emulsion of *M. melitensis* in normal salt solution. The dilutions of the serum were made with a mercury calibrated 5 cub. mm. pipette graduated in $\frac{1}{2}$ cub. mm., the various dilutions and the *M. melitensis* emulsion brought together on a glass slide, which was put in a moist chamber for 15 minutes and then examined under the microscope side by side with a control: and the highest dilution in which agglutination had by then occurred was recorded as the "maximum dilution of patient's blood giving agglutination reaction." It will thus be seen that all these are strictly comparable for both series. This was done for 89 cases out of the 103. It is usual to express a positive agglutination reaction for *M. melitensis* as 1 in "n," meaning that 1 bulk of serum in "n" bulks of normal saline effects agglutination: this may be expressed as a fraction $\frac{1}{n}$.

or again one may say that the agglutinating power of a given patient's serum is "n." In the following table in the column "Agglutinating power," the numbers given mean that one bulk of serum diluted with the corresponding number of bulks of normal saline has sufficed to produce agglutination under the conditions already specified; in the column headed "No *M. melitensis*" is placed the number of cases which did not yield *M. melitensis*; in the column headed "Recovery of *M. melitensis*" is placed the number of cases yielding *M. melitensis*, and in the last column are placed the minimal quantities of blood yielding *M. melitensis* for the specified agglutinating power.

Here one can trace no relation between the amount of agglutinating power and the presence or absence of *M. melitensis* in the blood, but there is some indication of a relationship between agglutinating power and minimal quantity of blood yielding *M. melitensis*, which might be tentatively put as follows:—The higher the agglutinating power of a

Agglutinating power.	No <i>M. melitensis</i> .	Recovery of <i>M. melitensis</i> .	Minimal quantity of blood yielding <i>M. melitensis</i> where recovered.	Agglutinating power.	No <i>M. melitensis</i> .	Recovery of <i>M. melitensis</i> .	Minimal quantity of blood yielding <i>M. melitensis</i> where recovered.
1 in 20	1			1 in 1080	2		
40	5	3	$\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$	1200	2	1	$\frac{1}{8}$
100	—	1	$\frac{1}{16}$	1400	1	2	1, 1
200	1	3	$\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$	1500	—	4	5, 1, $\frac{1}{4}$, $\frac{1}{16}$
300	—	1	1	1600	—	2	3, $\frac{1}{4}$
360	—	2	$\frac{1}{4}$, $\frac{1}{8}$	1800	3		
400	—	1	$\frac{1}{8}$	2000	1	7	5, 1, 1, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$
500	5	8	1, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$	2200	—	1	1
600	—	4	1, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$	2400	—	1	$\frac{1}{4}$
640	—	1	$\frac{1}{8}$	2500	1	4	1, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$
800	1	5	$\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$	2600	—	1	1
1000	2	8	2, 1, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$	3000	—	4	5, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$

blood during the fever, the larger is the minimal quantity of blood yielding *M. melitensis* likely to be; whence one might be tempted to deduce a correlation between high agglutinating power and high resistance to *M. melitensis* invasion on the part of the patient.

For how long is it Necessary to Incubate in Broth Patients' Blood containing M. melitensis before its Presence can be Demonstrated?

To obtain an answer to this question, the broth-tube containing the largest quantity of blood was, in 25 cases, used to inoculate, on each of eight successive days after its abstraction, an agar slope which was dated and incubated at 37° C. Seven of these cases failed to yield *M. melitensis*, in the others it made its appearance variably on the slope inoculated 3, 4, 5, 6, 7, or 8 days after commencement of the incubation of the blood in broth as follows:—

Number of days' incubation.	Number of recoveries.	Minimal amounts of blood ultimately yielding <i>M. melitensis</i> .
3	3	c.c. $\frac{1}{16}, \frac{1}{32}, \frac{1}{64}$
4	3	$\frac{1}{16}, \frac{1}{32}, \frac{1}{64}$
5	5	$\frac{1}{16}, \frac{1}{32}, \frac{1}{64}, \frac{1}{128}, \frac{1}{256}$
6	2	$\frac{1}{16}, \frac{1}{32}$
7	4	$\frac{1}{16}, \frac{1}{32}, \frac{1}{64}, \frac{1}{128}$
8	1	$\frac{1}{16}$

The general inference to be drawn from this is that, in general, the smaller the "minimal" amount of blood, the earlier *M. melitensis* makes a demonstrable appearance in the blood. The question is of some interest, because Widal, in his similar work on typhoid fever, postulated the hypothesis of the existence in the blood of typhoid cases in variable amounts of what he called "substances empechantes," which delayed the growth of *B. typhosus* in his nutrient broth according to the amount thereof; *B. typhosus*, in his experience, sometimes appearing after one day's incubation, in others not till after eight days. It will be observed that the foregoing cases yield some little support to the theory; for instance, in one case, where the minimal amount of blood was $\frac{1}{16}$ c.c., *M. melitensis* made its appearance after three days; in another case, where the minimal amount of blood was the same, $\frac{1}{16}$ c.c., not till the 7th day.

Diurnal Variation.

In 58 of the 103 cases blood was drawn in the forenoon; in 34 of these cases *M. melitensis* was present, in 24 it was absent; in 45 cases blood was drawn late in the afternoon; *M. melitensis* was present in 35 cases, absent in 10; a ratio of presence to absence of 7 to 5 in the

morning, as against 7 to 2 in the evening; that is *M. melitensis* was $2\frac{1}{2}$ times more likely to be found in blood taken in the late afternoon than in the forenoon; this suggests a correlation between the usually higher temperature of the patient in the afternoon and the presence of *M. melitensis* in his blood.

Summary.

1. *M. melitensis* has been demonstrated to be present in the peripheral blood of 68 per cent. (2 out of 3) of the cases examined in a series of 103 cases.

2. *M. melitensis* exists in the blood in relatively small amount, not having been found in association with a less quantity of blood than 4 cub. mm., and that only in two cases out of 103.

3. The higher the temperature of the cases for a few days before and at the period when blood is abstracted, the more likely is the latter to contain *M. melitensis*.

4. The higher the agglutinating power of a blood during the fever, the larger is the minimal quantity of blood yielding *M. melitensis* likely to be. A correlation is suggested between this and the patient's powers of resistance to *M. melitensis*.

5. The smaller the minimal quantity of blood yielding *M. melitensis*, the earlier is *M. melitensis* likely to be obtained from the nutrient broth in which it is being incubated.

6. *M. melitensis* is more likely to be present in blood abstracted in the late afternoon than in the forenoon. A correlation is suggested between this and usual evening rise of temperature.

7. No definite relation can be established between any chronological stage of the fever and the presence of *M. melitensis* in the blood; it is present both early and late in the disease.

II. ON THE INFECTIVITY OF THE SKIN, BREATH, AND SWEAT OF MEDITERRANEAN FEVER PATIENTS.

By Staff-Surgeon E. A. SHAW, R.N., Member Mediterranean Fever Commission, Malta.

1. *Examination of Skin.*

The epidermis is considerably affected in Mediterranean Fever. Hughes states that "about the fourth week desquamation occurs, being most noticeable on the soles of the feet, where the skin peels off in large flakes, leaving the skin of the feet soft and tender for a considerable time," "during the fourth month towards the end of long attacks or even during early convalescence, the hair falls out extensively." "In long cases the nails have often a grooved longitudinally striated appearance." Consequently, in March of 1904, I set myself to make an attempt to determine if *M. melitensis* is excreted in the cast-off epidermal scales of the skin. After some consideration I determined to put epidermal scrapings from Malta Fever Patients into nutrient broth, incubate this, and then from it to attempt to isolate *M. melitensis* if present.

As the skin is well known to be, even under apparently healthy conditions, largely infected with various organisms, and as any attempt at sterilisation thereof might kill off the somewhat delicate *M. melitensis*, and so baulk the object in view, it was felt that some other method of restricting the presence of organisms which would inevitably overgrow the slow growing *M. melitensis* during the process of isolation would have to be resorted to, and the addition of some antiseptic to the nutrient broth which would to some extent hold back the usual skin organisms, and yet not unduly check the possible *M. melitensis*, seemed the most promising method. Formalin was the antiseptic ultimately decided upon, and a series of preliminary experiments enabled one to determine that broth (made with peptone-Martin, and of an acidity + 5 Eyre's scale) containing 1 in 1000 of sodium formate would, after inoculation with *M. melitensis*, give a good growth thereof in four days; 2 in 1000 delayed growth till sixth day, which was felt to be too long, and less than 1 in 1000 would have defeated the object in view of restraining other organisms. The procedure finally adopted was the following:—

Some of the surface epidermis was removed from each selected Malta Fever patient with a sterilised scalpel by scraping the surface of the arms, chest, thighs, and feet; these scrapings were placed in a numbered, dated, test-tube containing the broth specified, incubated at

37° C. for five days (thus giving one day's margin), then the broth-tube was well agitated, a loopful taken from it and placed in another tube, of same number but new date, containing 10 c.c. sterile broth of the kind specified, this well agitated and mixed, and from this dilution zig-zag stroke inoculations were made on large agar (+ 5 Eyre's scale) slope tubes of same number and new date, the new tubes incubated for five days, the agar slopes examined daily for discrete colonies, which never failed to appear, and all of these resembling *M. melitensis* colonies were subjected to the usual tests; the second broth-tube used to form a similar dilution for third generation in broth and on agar slopes, and these again for Fourth Generation.

A total of 14 patients (the opportunity for obtaining material from whom I owe to the kind courtesy of the officers R.A.M.C. of Valletta and Cottonera hospitals, to whom I beg to tender my warmest thanks), all of which cases were undoubted Mediterranean Fever, ranging from three weeks to three months in duration of disease, were thus examined during April, May, and June, 1904. Every case yielded discrete colonies on the agar slopes, many of them greatly resembling *M. melitensis* colonies at first sight, but proving on further examination to be a white staphylococcus, apparently Welsh's skin staphylococcus, and not one of them turned out to be a colony of *M. melitensis*.

In August the foregoing method was modified as follows, the broth enrichment method being abandoned :—The epidermal scrapings from each patient were thoroughly ground up in 1 c.c. of sterile normal salt solution, one loopful of this was used to plate three successive glucose-litmus-agar Petri dishes; the remaining epidermal emulsion was diluted by the addition of 5 c.c. more normal saline, and the surface of three other similar Petris inoculated by spreading $\frac{1}{4}$ c.c. of this diluted epidermal emulsion over each, and the six plates then incubated at 37° for five days, at the end of which they were carefully examined for possible colonies of *M. melitensis*, and all likely looking ones put through the usual tests. A total of 71 specimens were thus examined. The accompanying table shows the cases and the day of disease on which specimens were taken, the sign x indicating each examination. The first 14 cases are not included, the day of disease not having been sufficiently accurately recorded.

From none of these bacteriological examinations has *M. melitensis* ever been recovered, but in nearly every plate out of the 426 used in this investigation has been found most constantly a Gram staining glucose fermenting white staphylococcus, presumably the same described by Welsh as associated with the skin, and on taking off the covers of the Petri dishes, a faint sour odour very similar to that noticeable on raising the bed-clothes of a feverish rheumatic patient was generally perceptible, suggesting the possibility of "sour sweats" being due to fermentations set up by this organism.

Animal Experiments with Skin Scrapings.

It was necessary to see also whether any evidence of the presence of *M. melitensis* in the skin of patients could be obtained by animal experimentation, and accordingly the following were undertaken:—

Experiment I.—Monkey No. 54.

A freshly arrived animal whose temperature and whose blood gave not the slightest indication of reaction to *M. melitensis* was set apart for this experiment.

July 21. Epidermal scrapings from the arms, forearms, and flanks of six Malta Fever patients, all between 28th and 35th day of disease, were taken, ground up in 5 c.c. sterile nutrient broth in a small sterilised mortar with a sterile pestle, and the resulting emulsion injected subcutaneously with a sterile syringe between the animal's shoulders, the intention being to first get general evidence, and later work out details.

July 22. Evening temperature had risen to 104° F.

July 24. Injection not yet showing any indication of absorption; persists as a globular swelling.

July 25. Some indication of commencing suppuration near site of injection. Evening temperature 104° F.

July 27. Commencing suppuration of 25th has now aborted. Injection still persists as a globular swelling.

July 29. Injectional swelling now disappearing.

July 31. No trace of agglutination in a dilution of $\frac{1}{20}$.

August 3. Temperature has remained normal since July 24. Again made emulsion of the skin-scrapings from five patients all between 30th and 45th day of disease, and injected subcutaneously.

August 5. No swelling, but some induration at sight of last injection.

August 7. No trace of agglutination reaction in a dilution of $\frac{1}{10}$. This monkey's box is next to that of Monkey No. 55, which, unknown to me, had been artificially infected with Malta Fever, and contact was possible (see note at end of this experiment).

August 9. Evening temperature to-day 104°, though normal from July 26 to this morning.

August 10. Agglutination reaction is present in a dilution of $\frac{1}{10}$ visible to naked eye, in $\frac{1}{20}$ visible under $\frac{2}{3}$ -in. objective.

August 11. Temperature again normal. Again repeated injection of epidermal scrapings from four other cases of Malta Fever, all between 30th and 45th day of disease.

August 15. Agglutination reaction present in a dilution of $\frac{1}{10}$ visible to naked eye, and of $\frac{1}{20}$ visible under $\frac{2}{3}$ -in. objective.

August 26. Agglutination reaction present as on August 15.

September 5. Agglutination reaction visible to naked eye in a dilution of $\frac{1}{20}$, and in $\frac{1}{40}$ under $\frac{2}{3}$ -in. objective.

September 28. Agglutination reaction as on September 5.

October 1. Agglutination reaction as on September 5. There has been no rise of temperature since August 9. Monkey killed with chloroform, an aseptic *post-mortem* made, and two broth-tubes and one agar slope inoculated with small cubes of tissue from the spleen, the broth-tubes each receiving a piece of the organ; similarly the liver and kidney, and all incubated. All organs were healthy, and there was no enlargement of the spleen.

October 8. No growth in any slope of October 1. Now inoculated six slopes from the six broth-tubes of October 1.

October 13. No growth in any slopes of October 1 or October 8. Experiment concluded.

Note.—As mentioned above, this monkey had been unwittingly exposed to the possibility of contact infection from Monkey No. 55, but as the animal cannot be safely said to have developed the fever, this does not matter. It had an occasional rise of temperature, but lasting only a day. No *M. melitensis* was recovered *post-mortem*, and an agglutination of $\frac{1}{40}$ alone is insufficient on which to base a diagnosis, and I should consider the development of this reaction due to the injection of *M. melitensis* toxins (contained in the skin). Of the action of toxins in producing the agglutination reaction I give experimental evidence in another section of this Report.

Experiment II.—Monkey No. 68.

During the first weeks of the last experiment the possibility of excretion of *M. melitensis* in the urine became an established fact, and as then the possibility of patients infecting the skin of their flanks with their own urine had to be considered, it was resolved that henceforth only scrapings from the upper arms of patients should be used. Further, the monkey (No. 68) used for this second experiment had his box put in a corner with no neighbour on his left, and the monkey used in the preceding experiment on his right, and as the latter did not develop the fever, and No. 68 was within reach of no other, he must be considered as not having been exposed to the risk of contact infection.

Monkey No. 68 was kept under observation from August 12 to 20; during this period his temperature varied from 100° — $103^{\circ}\cdot2$; blood presented no trace of agglutination reaction with *M. melitensis*.

August 20. Epidermal scrapings from upper arms of five patients, all between 30th and 60th day of disease, were emulsified as before, and injected between shoulders subcutaneously.

August 26. There has been no fever since last note, and to-day there is no trace of agglutination reaction.

August 27. Second injection of arm scrapings from two patients, both in 91st day of disease.

September 2. Third injection of arm scrapings of three patients, all between 60th and 90th day of disease.

September 5. Agglutination reaction present in a dilution of $\frac{1}{10}$, not beyond.

September 9. Ill, and off his feed.

September 10. Died between 5 and 7 P.M. In my absence an immediate *post-mortem* was made by Major Horrocks, who notes: "Very emaciated, maggots on skin of face, spleen and kidneys appeared slightly congested, other viscera appeared normal. Made cultures from spleen and kidney."

September 19. Agar slopes inoculated from spleen on 10th have remained sterile. Kidney slopes planted from broth on 14th also sterile; reinoculated these.

September 20. Reinoculated spleen slopes.

September 25. All remain sterile.

Result.—No development of fever, but development of a low ($\frac{1}{10}$) agglutination reaction, probably the result of injection of *M. melitensis* toxins in the epidermal scrapings. No *M. melitensis* recovered *post-mortem*.

Experiment III.—Monkey No. 62.

This experiment was commenced by Major Horrocks, and was turned over by him to me to complete on September 28, just prior to his departure for England. For convenience of comparison, I will briefly recapitulate Major Horrocks' notes.

Monkey No. 62 had had its blood frequently examined during August, and up to commencement of the experiment, and its temperature had been daily recorded. It was absolutely free from any suspicion of Malta Fever.

September 16. Monkey No. 62 received an injection of skin scrapings from arms and axilla of a fever patient, ground up in normal saline solution.

September 21. Blood examined, no agglutination reaction to *M. melitensis*.

September 24. Skin scrapings from same patient again injected.

September 26. Blood again examined. No agglutination reaction to *M. melitensis*.

September 27. Skin scrapings from same patient again injected.

September 30. This morning monkey was too sick to have his temperature taken. Died at 10.30 A.M. *Post-mortem* made at once. All organs seemed healthy. No cause of death discoverable. Inoculated broth-tubes and agar slopes from spleen, kidney, liver and heart's blood and incubated.

October 7. No growth on any slope of September 30. Incubated fresh slopes from broth-tubes of September 30.

October 12. No growth on any slope of October 7. Experiment concluded.

Result.—No development of Malta Fever. No development of agglutination reaction. No recovery of *M. melitensis*, *post-mortem*.

Note.—This experiment differs from the other two preceding it only in the non-appearance of the agglutination reaction; but, as in Experiment I, Monkey No. 54, the first skin injection was given July 21, and the *agglutination reaction* did not appear till August 10, an interval of 21 days, nor in Experiment II, Monkey No. 68, till after an interval of 16 days; nor in Experiment IV, Monkey No. 74, next to be described, till after an interval of 22 days; nor in Experiment V, Monkey No. 65, to be described next but one, till after an interval of 23 days; I think one is entitled to consider that as Monkey No. 62, Experiment III, died 14 days after its first skin injection, an interval shorter than any of those just cited, that there had not been time for the agglutination reaction as observed in the others, to develop.

Experiment IV.—Monkey No. 74.

This experiment, like the last, was commenced by Major Horrocks on September 12, during my absence, and turned over to me on September 28 to complete. Blood had prior to experimentation been frequently examined, but had never exhibited the slightest agglutination reaction with *M. melitensis*. There was never any possibility of contact infection.

September 12. Injection of emulsified skin scrapings from arms and axilla of one Mediterranean Fever patient.

September 17. Blood examined. Serum in a low dilution appeared to have a tendency to agglutinate *M. melitensis*.

September 23. Blood again examined. Serum in a dilution of 1 in 10 showed no sign of agglutinating *M. melitensis* after contact of one hour.

September 25. Second injection of skin scrapings emulsified in normal saline.

September 27. Third injection of skin scrapings.

September 28. Blood examined. No agglutination reaction.

October 3. Fourth injection of skin scrapings.

October 4. Blood gives a distinct agglutination reaction in a dilution of $\frac{1}{40}$ in 15 minutes visible under $\frac{2}{3}$ -in. objective.

October 8. Fifth injection of skin scrapings from three patients.

October 11. Blood gives distinct agglutination reaction in a $\frac{1}{30}$ dilution after 15 minutes visible to naked eye.

October 15. Sixth injection of skin scrapings from same three patients.

October 18. Agglutination reaction visible to naked eye in $\frac{1}{80}$ dilution.

October 19. Seventh injection of skin scrapings from three patients.

October 22. Eighth injection of skin scrapings from two patients.

October 25. Agglutination reaction in $\frac{1}{10}$ dilution visible to naked eye and in $\frac{1}{10}$ visible under $\frac{2}{3}$ -in. objective.

October 26. Ninth injection of skin scrapings from four patients.

October 28. This morning this monkey was found dead. *Post-mortem*. Stomach much dilated with gas. Organs all apparently healthy, much muscular wasting, abscesses at sites of injections. Broth-tubes and agar slopes inoculated from all organs and incubated.

November 2. Agar slopes of October 28 all sterile. Inoculated fresh agar slopes from broth tubes and incubated.

November 7. Agar slopes of November 2 sterile, as are also those of October 28. Experiment concluded.

Result.—Injection of skin scrapings has not been followed by fever, but has developed an agglutination reaction in the serum in low dilution appearing after an interval of 22 days from date of first injection.

Experiment V.—Monkey No. 65.

This monkey had been bitten in September by supposedly infected mosquitoes, but had never had any fever, nor had its blood, which with the others had been examined as a routine measure, once a week, ever shown any sign of agglutinating power on *M. melitensis*.

October 30. This monkey received an injection of epidermal scrapings from arms of four Mediterranean Fever patients.

November 3. Second injection of skin scrapings from four patients.

November 6. Third injection of skin scrapings from four patients.

November 7. Agglutination reaction = a slight tendency only visible in $\frac{1}{10}$, $\frac{1}{20}$ and $\frac{1}{40}$ dilutions, insufficient to call positive.

November 10. Fourth injection of skin scrapings from arms of four patients.

November 13. Fifth injection of skin scrapings from arms of four patients.

November 15. Agglutination reaction = nil in dilutions of $\frac{1}{10}$, $\frac{1}{20}$, and $\frac{1}{40}$.

November 16. Sixth injection of skin scrapings from arms of four patients.

November 19. Seventh injection of skin scrapings from arms of four patients.

November 22. Agglutination reaction distinct traces in $\frac{1}{10}$ and $\frac{1}{20}$ dilutions only.

November 24. Eighth injection of skin scrapings from arms of four patients.

November 27. Ninth injection of skin scrapings from arms of four patients.

November 28. Agglutination reaction marked in $\frac{1}{10}$ and $\frac{1}{20}$ dilutions.

December 1. Tenth injection of skin scrapings from arms of four patients.

December 4. Eleventh injection of skin scrapings from arms of four patients.

December 5. Agglutination reaction only in $\frac{1}{10}$ dilution visible under $\frac{2}{3}$ in. objective.

December 12. No agglutination reaction.

December 19. No agglutination reaction.

December 26. No agglutination reaction.

January 3. No agglutination reaction.

January 9. No agglutination reaction.

January 16. No agglutination reaction.

January 23. No agglutination reaction.

Experiment concluded. Monkey used for other experiments.

Result.—Infection of skin scrapings has not been followed by fever, but has developed in the blood a low agglutination reaction on *M. melitensis* after an interval of 23 days.

Remarks.—Summarising in tabular form the foregoing five experiments we get the following (see Table, p. 30).

In none of these monkeys can Malta Fever be said to have developed. The appearance of a low agglutination reaction alone is not sufficient on which to base a diagnosis and may, I think, safely be attributed to the presence of *M. melitensis* toxins in the skin scrapings used. It is to be noted that the highest dilution in which it was obtained was $\frac{1}{80}$; this is identical with the highest dilution which the agglutination reaction was obtained in Monkeys 58 and 59, which each received injections of the filtrate from broth in which *M. melitensis* had been cultured (*vide* toxin experiments), and both contrast markedly with the high dilution $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{3000}$, $\frac{1}{4000}$ in which agglutination is quite usually obtainable in monkeys which have received living *M. melitensis* in any experimental manner. The occasional elevations of temperature are attributable to the presence of staphylococci in the skin scrapings used, which had a decided tendency to produce abscess formation.

The point with regard to the toxins was felt to be an important one, and accordingly an effort was made to resolve it by an experiment which was commenced by Major Horrocks on September 22 and continued by me from September 28 to its conclusion on December 26.

Experiment number.	Monkey number.	Different samples of epidermis.	Number of injections.	Date of first injection.	Date of appearance of agglutination reaction.	Number of days required to develop agglutination.	Highest dilution which gave agglutination.	Post-mortem examination for <i>M. melleus</i> .
I	54	15	3	July 21	Aug. 10	20 days	$\frac{1}{10}$	Not recovered.
II	68	10	3	Aug. 20	Sept. 5	16 "	$\frac{1}{10}$	Not recovered.
III	62	3	3	Sept. 16	Never appeared. Died Sept. 30	Not recovered.
IV	74	18	9	Sept. 12	Oct. 4	22 days	$\frac{1}{10}$	Not recovered.
V	65	44	11	Oct. 30	Nov. 22	23 "	$\frac{1}{10}$	No post-mortem.

Experiment VI.

Monkey No. 61A. Had never had any elevation of temperature and had never presented agglutination reaction.

September 22. Skin scrapings from arms and axillæ of two Malta Fever patients were ground up with sterile normal salt solution into a fine emulsion. A sterile Berkefeld candle was fitted to a sterile test-tube, the emulsion filtered, and the filtrate injected subcutaneously into Monkey No. 61A.

September 24. Sweat obtained from three Malta Fever patients, similarly filtered and filtrate injected.

September 26. Blood presented no agglutination reaction.

October 3. Blood presented no agglutination reaction.

October 6. Injected filtered sweat from one patient.

October 8. Skin scrapings from three patients ground up in 15 c.c. sterile salt solution, allowed to macerate at laboratory temperature, 68° F., for two hours, filtered through Berkefeld candle and filtrate injected.

October 10. Blood presented no agglutination reaction.

October 15. Skin scrapings from three patients treated as on October 8 and filtrate injected.

October 17. Blood presented no agglutination reaction.

October 19. Skin scrapings and sweat from four patients treated as on October 8, and filtrate injected.

October 22. Skin scrapings and sweat from three patients treated as on October 8, and filtrate injected.

October 24. Blood presented no agglutination reaction.

October 26. Skin scrapings from four patients treated as on October 8, and filtrate injected.

October 30. Skin scrapings from four patients treated as on October 8, and filtrate injected.

October 31. No agglutination reaction.

November 3. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 6. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 7. Blood presented no agglutination reaction.

November 10. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 13. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 14. Blood presented no agglutination reaction.

November 16. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 19. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 21. Blood presented no agglutination reaction.

November 24. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 27. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 28. Blood presented no agglutination reaction.

December 1. Skin scrapings from four patients treated as on October 8, and filtrate injected.

December 4. Skin scrapings from four patients treated as on October 8, and filtrate injected.

December 26. Blood has been examined weekly since last note and also to-day and has never presented any agglutination reaction with *M. melitensis*.

Experiment concluded.

Remarks.—This experiment, so far as it goes, would appear to negative the explanation given of the appearance of the agglutination reaction in four out of the five preceding experiments, but it is to be observed that it takes for granted one important point, *i.e.*, that *M. melitensis* "toxins" are soluble in normal salt solution. This point is reserved for future experiments, as it does not affect the main inference to be drawn from this series of experiments.

Conclusion.—The active infective agent of Mediterranean Fever is not excreted by the skin.

2. Examination of Breath.

With a view to obtaining experimental evidence on this question it was determined to instruct patients to gently blow through sterile broth contained in sterile tubes and examine this broth bacteriologically. Broth tubes were fitted with rubber corks bored with two holes, through one of which was passed a long glass entry tube, bent outside the broth-tube at an obtuse angle of about 150°, length outside broth-tube being about 3 inches, inside dipping beneath surface of broth; through the other hole in the rubber cork was passed a short straight glass air-exit tube lightly plugged with sterile cotton wool 1-inch in length on each side of the rubber cork. The patients whose breath it was desired to examine, were instructed to blow gently down the long entry tube at frequent intervals during one hour, their expired air gently bubbling through the broth. In experiments made before the present series the entry tube outside the broth-tube was fitted with a longish piece of rubber tube and a glass mouth-piece, but it seemed to me that if expired air contained any microbes there was great risk of these being caught by the moist inner surface of the rubber tube, on which a considerable proportion of the water vapour contained in all expired air was found to condense.

First Method.—These broth-tubes on arrival at the laboratory had

their rubber corks and glass tubes removed, were replugged with sterile cotton wool, incubated for seven days at 37°, and then one loopful from each distributed over the agar surfaces of two large-sized Petri dishes, which were incubated for five days at 37°, and then examined for individual *M. melitensis* colonies. A total of 86 such breath-tubes were examined in the manner described, but *M. melitensis* never appeared in any plate, though other organisms, not examined in detail, did.

Second Method.—At this stage other experiments on the vitality of *M. melitensis* growing with other organisms (see Section on Vitality of *M. melitensis* outside the Body, p. 43) had shown that *M. melitensis* had not much chance of surviving in a fluid nutrient medium with other microbes. Accordingly from each broth-tube immediately on its arrival at the laboratory, after well shaking, a loopful was taken and distributed over the agar surface of two Petri dishes, the tubes then plugged with sterile cotton wool and both tubes and plates incubated. At the end of five days these “direct” plates were carefully examined for *M. melitensis*, and at the end of seven days’ incubation a loopful from each breath broth-tube was distributed over two agar plates and these also examined after five days’ incubation for possible *M. melitensis*. One thus had two series of plates, one direct, the other after the incubation of the breath-infected broth. A total of 24 breath-tubes were treated in this manner, but no *M. melitensis* ever appeared on any plate, though the same type of other organisms as before were found.

Third Method.—By this time experiments with *M. melitensis* in association with other organisms had shown that a period of three days was as long as one could expect to recover *M. melitensis* when incubated in broth with other organisms. Accordingly the “direct” series of plates was continued as before, but the breath broth-tubes were only incubated for three days and then plated.

I examined 115 breath broth-tubes in this way, but not in one did I find a single *M. melitensis* colony.

A total of 225 such broth-tubes were examined, and in connection therewith 728 agar Petri plates were prepared and examined, all without result so far as the particular quest involved was concerned. I append a table showing names of patients and day of disease in each case in which breath broth-tubes were prepared, which is indicated in each case by the sign ×. It will be seen that practically the whole period of the disease has been covered.

In order to further investigate the possibility of the infection of Malta Fever being given off in the breath, animal experimentation was also resorted to; portions of such of the foregoing broth-tubes as presented growth being injected in quantities usually of 10 c.c. into two monkeys. This was commenced in the first monkey, No. 73, by Major Horrocks, September 16, and continued by me from September 28 till this monkey's death on November 1; in the second monkey, No. 43, commenced and concluded by me alone.

Monkey No. 73.—This animal had never been used for any other experiment.

September 15. Blood examined. No reaction to *M. melitensis*.

September 16. Injected contents of broth-tube infected by Malta Fever patient's (Lawrence) breath in which growth had occurred after incubation.

September 21. Similar injection (Silburn).

September 28. Blood examined. No reaction to *M. melitensis*.

October 3. Big abscess at site of former injections. To-day injected a growth in breath broth-tube (Anderson) on opposite side.

October 4. Blood examined. No reaction to *M. melitensis*.

October 6. Injected broth growths from breath of Rentcome and Marchant, 5 c.c. from each.

October 9. Similar injection (Silburn).

October 11. Blood examined. Agglutination reaction with *M. melitensis* in $\frac{1}{10}$ dilution under $\frac{1}{2}$ -in. objective.

October 12. Injected broth growths from breaths of Campbell, Joyce and Silburn, 3 c.c. each.

September 16. Injected broth growths from breaths of Campbell, Grimwood and Kinsella, 3 c.c. each.

October 18. Blood presents agglutination reaction in a dilution of $\frac{1}{100}$ visible to naked eye.

October 19. Injected broth growth from breaths of Campbell and Grimwood, 5 c.c. of each.

October 23. Injected broth growths from breaths of Campbell, Kinsella, and Joyce, 3 c.c. of each.

October 25. Agglutination reaction in a dilution of $\frac{1}{100}$ visible to naked eye.

October 26. Injected broth growths from breaths of Fletcher, Groom, Russell and Tait, $2\frac{1}{2}$ c.c. from each.

October 29. Monkey ill. Considerable diarrhoea and wasting.

November 1. Monkey obviously dying. Euthanasia cum chloroform. *Post-mortem.*—Much wasting, no obvious cause of death, organs all apparently healthy, gas in intestines. Agar slopes and broth-tubes inoculated from all organs and incubated.

November 6. Agar slopes of November 1 all sterile. Inoculated fresh slopes from broth-tubes of November 1.

November 11. Agar slopes of November 6 also sterile.

Experiment concluded.

Monkey No. 43.—This animal, in July, on the 16th and 18th, had received injections of the filtrate through filter paper of supposedly infected soil macerated in sterile water, but had never developed Malta Fever, nor had its blood ever reacted to *M. melitensis* in any dilution whatever, though frequently examined.

October 3, 10, 17, 24. No trace of agglutination reaction in dilutions of $\frac{1}{10}$, $\frac{1}{20}$, or $\frac{1}{40}$.

October 27. Injected broth growth from breaths of Grimwood, Joyce and Silburn, 3 c.c. from each.

October 31. Injected broth growth from breaths of Donovan and Silburn, 5 c.c. from each. No agglutination reaction in $\frac{1}{10}$, $\frac{1}{20}$ or $\frac{1}{40}$ dilutions.

November 4. Injected broth growth from breaths of Groom and Silburn, 5 c.c. each.

November 7. A doubtful tendency to agglutination in $\frac{1}{10}$, $\frac{1}{20}$ dilution under $\frac{1}{2}$ -in. objective. Abscess at the site of last injection but one.

November 8. Injected broth growth from breaths of Turner and Kinsella, 5 c.c. each.

November 12. Monkey somewhat ailing.

November 14. No agglutination reaction.

November 17. Injected broth growth from breath of Grimwood, 10 c.c.

November 21. Injected broth growth from breaths of Dennis and Grimwood, 5 c.c. each.

November 21. No agglutination reaction.

November 28. Slight agglutination reaction in $\frac{1}{10}$ and $\frac{1}{20}$ dilutions under $\frac{2}{3}$ -in. objective.

December 1. Injected broth growth from breaths of Darby and Walker, 5 c.c. each.

December 4. Injected broth growth from breaths of Darby, Walker, and Turner, 5 c.c. each.

December 5. Tendency to agglutination in $\frac{1}{10}$ dilution.

December 12. No agglutination reaction. Very weak, thin and emaciated; has been seedy for some days.

December 15. Dying. Gave chloroform. *Post-mortem*, found pneumonia and pericarditis left side. Inoculated slopes and broth-tubes from all organs.

December 23. No *M. melitensis* recovered *post-mortem*, but a glucose fermenting + Gram-staining coccus was obtained from spleen, liver and kidney; nothing from heart's blood and lungs.

Remarks.—There is to be noticed in both these animal experiments the development of a low agglutination reaction, and here as in the

skin experiments I should attribute this to the ingestion of *M. melitensis* toxins, as I consider it practically certain that in breathing out through the broth-tubes, a certain amount of saliva trickled down the long entry tube and so into the broth; the possibility of this was considered at the time and efforts were made to arrange some method of passing breath containing possibly *M. melitensis*, while excluding saliva; but none free from objection was found, hence it was decided to proceed as described, and if *M. melitensis* were obtained, to examine the saliva of patients independently for this micro-organism. In neither monkey could it be said that Mediterranean Fever was developed. The agglutination reaction developed was much too low, the occasional rises of temperature observed were attributable to abscess formation, or to other micro-organisms, not *M. melitensis*, contained in the broth growths which were injected. In both cases temperatures were taken morning and evening all through the experiments.

3. *Examination of Sweat.*

Critical sweats are a not infrequent and quite characteristic feature of Mediterranean Fever, and it has been often felt that it was not impossible that the *Micrococcus melitensis* might be passed out of the body in this secretion. To determine this exhaustively, I made a bacteriological examination of 251 specimens of sweat obtained from patients in the Military Hospital at Valetta. The method adopted was varied from time to time, as will be described.

First Method.—A skin surface of forearm washed with spirit soap, then ether, a carbolic pad 1 in 40 kept on 12 hours, then a circle of sterilised (dry, 160° C. in air) lint, placed on this surface, and a sterilised watch glass strapped over it with adhesive plaster. After critical sweating, circle of lint removed, placed between two sterilised watch glasses held in a metal frame, and sent to me at laboratory. There each circle of lint placed in a separate broth-tube numbered, dated, and incubated at 37° C. After five days' incubation, agar slopes inoculated zig-zag from each, were incubated at 37° C., and examined daily for growth; if sterile, original broth-tubes were inoculated with *M. melitensis*, returned to incubator for four days, and then fresh slopes inoculated from them; on these *M. melitensis* invariably appeared, thus proving that sufficient disinfectant to prevent growth of *M. melitensis* had not been carried into circles of lint from disinfection of skin surface.

Nineteen sweat swabs from different patients were thus examined. In some cases the tubes remained sterile, in others the agar slopes yielded growth in discrete colonies.

Result.—No *M. melitensis* was ever recovered by this method.

Second Method.—The critical sweat was collected in sterile pipettes from four different patients, zig-zagged on agar and incubated. The

collection was done by the sisters in the ward, who were supplied with the pipettes ready for use, and instructed how to break the point and apply them. They stated it was rare for sweat to collect in such large drops as to admit of collection in this manner, hence specimens were obtained from only four patients.

Result.—No *M. melitensis* was obtained.

Third Method (a modification of the first).—Circles of lint were obtained saturated with critical sweat from Malta Fever patients, as in first method, but instead of being incubated in broth-tubes, were placed each in a 5 c.c. sterile normal salt solution tube, in which they were thoroughly agitated and ground up with a sterile glass rod, and the resulting fluid plated out in agar Petri dishes both by spreading $\frac{1}{2}$ c.c. of it over whole surface, and by describing a centripetal spiral with a loopful of the fluid. Discrete colonies were always thus obtained after incubation at 37° C. The critical sweat of seven patients have been thus examined without *M. melitensis* having been obtained.

Fourth Method.—The circles of lint saturated with sweat obtained as in the first method were each placed in a separate broth-tube which was incubated at 37° C. for seven days; then a loopful was taken and placed on the surface of nutrose-glucose-litmus-agar in a Petri dish and spread over it by means of a Klein's platinum spreader, which, after completely going over the agar surface, was straightway passed over the surface of a second similar Petri plate. These plates were then incubated for five days, after which they were examined carefully for possible *M. melitensis* colonies. A total of 81 specimens were thus examined.

Result.—No *M. melitensis* were recovered.

Fifth Method (a modification of the third).—It seemed not unlikely that, supposing *M. melitensis* to be present in the circles of lint saturated with critical sweat, that it would be more likely to be obtained directly without previous incubation if nutrient broth were used instead of the salt solution, specified in Method 3; so, accordingly, the circles of lint were placed in separate broth-tubes, and thoroughly stirred up and agitated therein with a sterile Klein's spreader, followed by a vigorous shaking. Then one platinum loopful was immediately spread over an agar plate and incubated for five days at 37°, and then examined for possible *M. melitensis* colonies. The broth-tubes containing the sweat-saturated lint were incubated seven days, and then one loopful spread over two agar plates. These similarly incubated five days and then examined.

A total of 24 specimens were thus treated, but no *M. melitensis* was ever obtained on either series of plates.

Sixth Method.—At this stage of the examination the results of other experiments (see Section on Vitality of *M. melitensis* outside the Body) indicated that *M. melitensis* could not be expected to be recovered

after three days in a nutrient medium containing other organisms; so consequently the procedure of the fifth method was modified by reducing the period of incubation of the sweat-saturated lint in broth to three days instead of seven, being otherwise identical.

A total of 30 specimens were so examined, but again no *M. melitensis* was ever obtained from either the plates inoculated the day the specimen was received, or from those inoculated from the broth-tubes after three days' incubation.

Seventh Method.—Instead of grinding up and shaking the sweat-saturated piece of lint in nutrient broth, and immediately plating a loopful of this, the piece of lint as received was placed flat on the agar surface of a Petri dish and pressed well on to the agar with a sterilised Klein's spreader, then it was lifted up with a pair of sterilised forceps and removed with the same surface downwards to the next adjacent area of agar, and there again pressed on to the agar; this process was repeated until the whole of the agar surface of the Petri dish was covered with "impressions" made from one surface of the piece of lint, usually 30 to 40 for a 10-centimetre Petri. Now another agar Petri was taken, and the same process repeated, but with the other surface of the lint, and both Petri dishes put in incubator at 37° C.; this completed, the piece of lint was then put in a broth-tube and incubated three days at 37° C., and then one loopful plated over two agar Petris.

A total of 86 specimens were thus examined, but no *M. melitensis* was ever obtained either from the direct series of "impression" plates or from the broth-tubes containing the circles of sweat-saturated lint. Frequently in the course of the examination one met with the colonies of *plus* Gram-staining glucose fermenting staphylococci, which turned up almost invariably in these bacteriological examinations of the skin.

The accompanying table (pp. 41, 42) shows the patients and the day of disease on which a specimen was taken; it will be seen that practically every day of the disease is represented by one or more examinations. The numbers on the top line indicate the day of disease, one column being given to each; the sign × indicates that an examination was made, being placed in the vertical column of the day of disease, and in the horizontal column appropriated to the name of the patient from whom it was taken.

It was found practically impossible to obtain sweat in such quantity as to admit of satisfactory injections into animals, but the number of specimens (251) examined, covering every period of the disease, and the varying methods employed, some of which succeeded so admirably in the isolation of *M. melitensis* from the blood and the urine of patients, practically justify the assumption that *M. melitensis* is not excreted in sweat of Malta Fever patients, or it would have been recovered in one of these numerous attempts.

Result.—*M. melitensis* has not been recovered from the 251 specimens of sweat examined, and in all probability is not excreted in this secretion.

Table showing Cases and Day of Disease on which Sweat was Bacteriologically Examined—*continued.*

Patient's name.	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	
Pigott.....																																															
Wilson	x																																														
Hagger																																															
Kelly																																															
Marham																																															
Mayes																																															
Francois																																															
Lawrence																																															
Martin																																															
Jones																																															
Hewett																																															
Pudney																																															
Curry																																															
Vincent																																															
Rivers																																															
Hurrell																																															
Anthony	x																																														
Marchant	x																																														
Silburn	x																																														
Kinsella	x																																														
Turner	x																																														
Anderson	x																																														
Dennis	x																																														
Derby	x																																														
Ericson																																															

III. ON THE VITALITY OF THE *MICROCOCCUS MELITENSIS* OUTSIDE THE BODY IN DIFFERENT ENVIRONMENTS.

By Staff-Surgeon E. A. SHAW, R.N., Member Mediterranean Fever Commission, Malta.

In attempting to ascertain the presence or otherwise of *M. melitensis* in the skin, sweat, breath, etc., of patients suffering from Malta Fever, it was obviously of some importance, having regard to the slow growth of this organism, as compared with the rapid growth of others in nutrient broth, a medium which could not be dispensed with, to ascertain for how long it could be recovered when incubated in broth in association with other micro-organisms, and accordingly the following experiments were undertaken, a control inoculation of the *M. melitensis* used being made into a tube of the same broth to verify its viability, the same generation of *M. melitensis* being used throughout.

A. *Vitality of M. melitensis in Mixed Broth Culture.*

No. 1. A sterile broth tube was inoculated with *M. melitensis*, and also from a broth tube (similarly with the same platinum loop) which had been allowed to become contaminated by exposure in the laboratory. This tube was well shaken and then one loopful was distributed over the surface of three successive agar Petri dishes with a Klein's spreader, and then tube and plates placed in incubator at 37°; each day another series of three plates was similarly inoculated from the broth tube, which was each time returned to the incubator, and after a period of five days' incubation, each set of three Petris was carefully examined for *M. melitensis*. This was found in the plates inoculated from the mixed broth culture after it had been incubated seven days, but not later.

No. 2. A repetition of No. 1. The result was the same. *M. melitensis* was recovered from the mixed culture for seven days, but no longer, in both cases the mixed culture at the termination of the experiment, 15th day of incubation, was slightly alkaline to litmus.

No. 3 and 4. On the same lines as No. 1; but in these two the mixed culture was composed of *M. melitensis* plus organisms derived from sweat, skin, and urine of Malta Fever cases, as far as possible equal quantities of each being taken. In No. 3, *M. melitensis* was recovered after two days' incubation, not later. In No. 4, started a week later, but with skin, sweat, and urine organisms from different sources, *M. melitensis* was not recovered at all. In both the reaction of the

mixed broth culture was acid to litmus at the termination of the incubation (seven days).

Nos. 5, 6, 7, 8, 9, and 10. In each of these, performed successively, not collectively, the same procedure as in No. 1 was followed, the organisms used being *M. melitensis*, and cultures derived from skin, breath, and sweat. In none was *M. melitensis* recovered after more than one day's incubation, and in all the mixed broth culture was acid to litmus at the end of each incubation.

Result.—*M. melitensis* incubated in broth in presence of other organisms is recoverable for a very short time, seven days, in presence of alkali producing organisms. One to two days in presence of acid-producing organisms contrasting greatly with its recoverability in pure broth culture, from a tube of which, inoculated December 12, 1904, it was recovered by a sub-culture on agar, April 25, 1905, an interval of over four months.

B. *Vitality of M. melitensis in Pure Culture.*

1. *On agar agar dry.*—Two agar slopes inoculated with *M. melitensis* March 29, 1904, which had been incubated at 37° C. for four days and then placed aside in laboratory cupboard with cotton wool plug unprotected by a rubber cap, had on December 30, 1904, become so dry that no colony could be detached for sub-culture, and the agar itself had contracted to a thin shred; sterile broth was therefore added to the two tubes until the upper level of the dry culture was submerged, these were then placed in the incubator at 37° C. till January 4, when the broth of one had become turbid, this was now sub-cultured, and pure *M. melitensis* was recovered and verified. No growth was obtained from the other.

Two similar slopes inoculated April 5, 1904, examined January 24, 1905, failed to give any growth. Two other such slopes inoculated April 21 and 24, 1904, similarly examined March 20, 1905, failed to give any growth.

Result.—*M. melitensis* had remained alive and capable of reproduction in a dried-up condition on agar from March 29 to December 30 = 276 days (nine months).

2. *In Litmus Milk.*—A tube of litmus milk inoculated with *M. melitensis* (Second Generation, Human Spleen, Bowles), December 12, 1904, yielded *M. melitensis* in sub-culture April 26, 1905, a period of over four months, but in very small quantity, a loopful which in January and February had yielded colonies by the hundred, now giving only 1 to 10 colonies; and after May 5 ceased yielding colonies though experimented with for a fortnight longer, hence, presumably dead after 144 days of vitality.

3. *In Nutrient Ordinary Beef-Peptide Broth.*—A tube of this was inoculated from same source at same time as litmus milk, December 12,

1904, and right up to June 3, 1905, each loopful taken twice weekly for sub-culture was yielding a plentiful supply of *M. melitensis*.

Result.—Still alive and actively reproductive after 5½ months in nutrient broth.

In all these the media were titrated to a reaction of + 10 of acidity with phenol-phthaleine (Eyre's scale).

Results.

<i>M. melitensis</i> lived on dry agar	276 days.
" " in litmus milk	144 "
" " nutrient broth.....	173 "

C. Vitality of M. melitensis in Urine.

A noteworthy feature of the urine of Mediterranean Fever patients is the length of time it remains acid after it has been passed; the following observations given in tabular form demonstrate this. These urines were taken from patients in the wards without special precautions and were kept in the laboratory cupboard, again without any special precautions. The acidity was determined each time by titration against a standard $\frac{N}{5}$ solution of potassium hydrate, phenol-phthallein being used as the indicator, and is expressed according to Eyre's scale. It will be remembered that the optimum reaction of culture media for *M. melitensis* has been found to be an acidity of + 10, Eyre's scale (see Part I of these reports).

Patient's name.	Date urine passed.	Acidity when passed.	Acidity on Jan. 30.	Acidity on Feb. 21.	Acidity on Mar. 29.
Anderson	Jan. 3	+ 36		+ 36	+ 26
Turner.....	3	+ 5		+ 2	- 2 alkaline
Martin.....	3	+ 44	+ 44	+ 40	+ 18
Rentcombe (a) ...	3	+ 60		+ 28	+ 4
"	12	+ 30		+ 12	+ 12
" (b)	15	+ 52	+ 40		
Webb (c).....	Feb. 5	+ 60			+ 50
Jacombe (d)	5	+ 32			+ 32

In the following observations on the life of *M. melitensis* in various specimens of urine (again given in tabular form), the following was the method adopted. The specimens of urine were some healthy and some obtained from Malta Fever patients, and one of each sterilised in autoclave at 115°. In each case, after the acidity of the specimen had been determined, 10 c.c. of it were placed in a sterile test-tube with the usual wool plug. As it was also considered of importance to have information as to the number of colonies of other micro-organisms

these specimens contained, each was well shaken and one loopful, taken with a standard loop, distributed over the surface of an agar Petri dish, which was then incubated at 37°, and the number of colonies counted and recorded five days later. After the abstraction of this one loopful, each specimen was inoculated with *M. melitensis*. The same brand was placed in each, a four days' growth on glucose litmus agar of the second generation of *M. melitensis* obtained from the spleen of a fatal case (Bowles) and, as far as possible, the same amount of culture in each case, a small platinum loop being set aside for the purpose of delimiting each time the area of agar to be denuded of growth. The tubes of *M. melitensis* inoculated urine were now placed in the laboratory cupboard (temperature about 15° C.) and daily well shaken and a loopful from each plated on agar, the plate incubated for five days and then examined for *M. melitensis*, which, if found, was verified in the usual way. It was found that after a variable number of days, some morning, a plate from a given urine would contain no *M. melitensis*: this was regarded not as a sign of death of all the *M. melitensis* in the specimen, but as indicating a great diminution in number, and the daily plating persevered with till there had been a succession of seven blank days. Not infrequently a specimen would yield *M. melitensis* one day, then not yield it for one or two days and then again give it. After a succession of seven days' plating without recovery of *M. melitensis*, that particular observation was terminated with the then acidity of that particular specimen being determined and recorded.

The following were the results obtained :—

Source of urine used.	Reaction.	No. of colonies per loop.	No. of days <i>M. melitensis</i> was recovered.	Reaction of urine at end of observation.
Unsterilised, normal healthy ...	+ 7 acid	57	2	Very alkaline.
" " " ...	+ 8 "	85	5	Just neutral.
" " " ...	+ 7 "	57	2	Alkaline, strongly.
" " " " ...	+ 8 "	5	33	- 5 alkaline.
Unsterilised, Malta Fever patient	+ 18 "	13	15	-40 "
(a) " " " "	+ 60 "	32	24	- 25 "
(b) " " " "	+ 52 "	7	18	- 25 "
" " " " "	+ 40 "	6	43	Just neutral.
(c) " " " " "	+ 60 "	175	36	+ 16 acid.
(d) " " " " "	+ 32 "	51	49	+ 4 "
Sterilised, normal healthy.....	+ 8 "	Nil	17	+ 2 "
Sterilised, Malta Fever patient...	+ 40 "	"	33	+ 20 "

A control inoculation of the same culture of *M. melitensis* into nutrient broth, kept under same conditions, was recovered by sub-culture on to agar after 4½ months.

Remarks.—Here neither differences in acidity or in number of other organisms seem to have had an appreciable influence on the duration of life of *M. melitensis* in urine, variations in this being presumably due to variations in other constituents so far as urine derived from Malta Fever patients is concerned, though there does seem to exist a direct connection between duration of life of *M. melitensis* and number of other organisms in the case of normal healthy urine, the greater the number of the latter the shorter the life of *M. melitensis* in such urine containing both. The urines lettered (a), (b), (c) and (d) in the two Tables I and II were identical, and the date of last determination of acidity in Table I was after the final one in Table II. The factor of difference was the presence of *M. melitensis* in urines of Table II and its absence in those of Table I, and I think the development of alkalinity in the urines of Table II compared with its non-development in the identical urines of Table I is attributable to the presence of *M. melitensis* in those of Table II, this being in accord with other observations on production of alkalinity by *M. melitensis* in nutrient media (see Part I of these reports).

The salient feature, however, is the comparatively long retention of reproductive activity of *M. melitensis*, lasting as long as seven weeks, in the urine of Mediterranean Fever cases. I may remark here that I find that 23 out of 30 samples of urine from Mediterranean Fever cases examined effected agglutination of *M. melitensis* in varying degrees; evidently agglutinins are excreted in the urine.

D. *Vitality of M. melitensis in Diluted Urine.*

1. The same brand and quantity of *M. melitensis* was placed in 1 c.c. of fresh healthy urine, which was well shaken and then added to 100 c.c. of sterilised tap water contained in a flask with cotton wool plug, the idea being to simulate the diluted fluid of the ordinary urinal minus accessory contaminations. Here again the flask was daily well shaken and a loopful plated on agar, the plate incubated and examined for *M. melitensis* colonies in the usual way. *M. melitensis* was recovered for nine days.

2. The same experiment was repeated, using urine from a Malta Fever case, and *M. melitensis* was recovered day by day, with occasional intervals of one, two, or three days, for 79 days; the daily sub-culture was persevered with for 14 days longer without *M. melitensis* being recovered.

Result.—1. *M. melitensis* was recovered from diluted healthy urine for nine days.

2. *M. melitensis* was recovered from diluted Mediterranean Fever urine for 79 days.

E. Vitality of M. melitensis in Urine—Contaminated Milk.

1. The same brand and quantity of *M. melitensis* was placed in 1 c.c. fresh healthy urine, which was well shaken and then added to 100 c.c. of sterilised goat's milk contained in a wool-stoppered flask, which was thoroughly well shaken every morning, and then a loopful plated on agar, incubated and examined for *M. melitensis* colonies. *M. melitensis* was recovered for three days, but after that was completely crowded out by other colonies.

2. The same experiment was repeated, using *Malta Fever* urine instead of healthy urine. In this case *M. melitensis* was recovered for 38 days.

Result.—1. *M. melitensis* recovered from milk contaminated with healthy urine for three days.

2. *M. melitensis* recovered from milk contaminated with *Mediterranean Fever* urine for 38 days.

F. Vitality of M. melitensis in Urine Dried on Fabrics.

The intention was here to obtain information as to the possible infectivity of garments soiled with urine containing *M. melitensis*.

1. Ten c.c. of normal healthy urine were taken and inoculated with the same brand and quantity of *M. melitensis* as in the preceding urine experiments, pieces of sterile lint were immersed in it till saturated, then removed and allowed to dry in a sterile Petri dish at the laboratory temperature (about 15° C.), which took four days. Then daily two small pieces were snipped off with sterile scissors, one put in a 10 c.c. broth tube, the other used to make impressions on the surface of agar in a Petri dish, by lifting it from area to area of the agar with a pair of forceps, and in each new situation pressing it on to the surface of the agar with a platinum spreader. The broth-tube and plate were then incubated and examined for *M. melitensis* in the usual way, but in this experiment none were recovered.

2. Precisely the same experiment, but using navy blue serge No. 3, such as is worn by the bluejacket. Again the result was the same, no *M. melitensis* was recovered.

3. Thinking that the failure to recover *M. melitensis* in the two preceding experiments might be due to the very slow drying on the fabric and the consequent facility for fermentation of the urine which certainly took place, the same experiments were repeated, using *Malta Fever* urine and drying the fabrics in the incubator at 37° C.; this was found to take only 24 hours instead of the four days requisite at atmospheric temperature.

Result.—*M. melitensis* was recovered from the lint so treated for five days, and from the blue navy serge for 78 days; daily sub-inoculations having been made as described in F 1. The difference between the

periods of recovery in the two cases may doubtless be attributable to the very different modes of manufacture of the two fabrics.

G. Vitality of M. melitensis in Sterilised Tap Water.

Ten c.c. of ordinary tap water were taken in a test-tube with cotton wool plug and sterilised in autoclave at 115° and then inoculated with same brand and quantity of *M. melitensis* as in the urine experiments, C 2, and placed in laboratory cupboard at temperature of about 15° C. Each day this tube was well shaken and cultured as described in C, by means of standard platinum loop and spreader on agar in Petri dishes.

Result.—*M. melitensis* was recovered for 50 days.

H. Vitality of M. melitensis in Unsterile Tap Water.

The same experiment as G, but the tap water not sterilised and two observations were made.

1. Commenced December 12, finished December 29. *M. melitensis* recovered for 10 days, tap water from rain tank on roof being used; this is not usually considered potable.

2. Commenced December 30, finished March 23. *M. melitensis* was recovered for 72 days, tap water from ordinary urban house-supply being used, which is used for drinking purposes.

Result.—1. *M. melitensis* was recovered from tank water for 10 days.

2. *M. melitensis* was recovered from potable water for 72 days.

I. Vitality of M. melitensis in Unsterile Sea Water.

This experiment was made twice in the same way as those described under G and H, the same brand and quantity of *M. melitensis* being used. The sea water was obtained from the area of the Grand Harbour in which H.M.S. "Egmont" (a stationary dépôt ship on board of which a varying number of 300 to 600 men are living) is moored, and in close proximity to this ship, with the sewage of which it was demonstrably fouled.

Result.—1. The first specimen of sea water yielded three colonies per loop of other micro-organisms before inoculation, and *M. melitensis* was recovered from it after inoculation for 46 days.

2. The second specimen of sea water yielded 11 colonies per loop of other micro-organisms before inoculation, and *M. melitensis* was recovered from it after inoculation for 11 days.

J. Vitality of M. melitensis Dry on Cover Slips.

A large number of cover slips were cleaned and sterilised, an emulsion of same brand of *M. melitensis* as that used in all the preceding experiments made in sterilised distilled water, and one drop

of this placed with standard platinum loop on one surface of each cover slip, these being arranged in rows inside sterile Petri dishes wherein the drops of emulsion were allowed to dry, the whole being kept in laboratory cupboard at about 15° C. Each day one was removed with a pair of sterile forceps and placed, *M. melitensis* film-side downwards, on the surface of agar contained in a Petri dish, over which it was moved by the forceps till all the *M. melitensis* film had apparently been left distributed over the agar surface, when it was left *in situ*, still with its "film" side adhering to the agar, the cover of the Petri replaced numbered and dated, and the whole incubated for five days and then examined for growth.

Result.—*M. melitensis* was recovered in this way from these cover slips for 15 days; a result of some importance as showing the inherent vitality of *M. melitensis* even when dried and separated from any trace of organic matter. It will be remembered that in the dry condition on organic matter (nutrient agar) it lived for over nine months (*vide* Section B of these experiments).

K. *Vitality of M. melitensis in Earth.*

1. *In Sand Free from Organic Matter.*—The sand used was a silicious red sand obtained from North Africa; it was heated to redness to burn off organic matter, then well shaken with distilled water, the reaction of which was after this found to be neutral. Some of it was then sterilised in dry air at 160° C. inside a pair of watch glasses held in a clip. An emulsion of the same quantity and brand of *M. melitensis* as in preceding experiments (Second Generation from Human Spleen, Bowles) made in 5 c.c. of distilled sterilised water and well mixed with the sterilised sand, and the whole allowed to dry in a cupboard at the temperature of the laboratory, 15° C. Twice a week two specimens were put out to incubate as follows: (a) A little of the inoculated sand was put in a 10 c.c. broth tube which was then incubated for five days, after which a loopful was put out on a glucose-litmus-agar slope which was incubated and examined for growth; (b) A little was put on the surface of similar agar in a Petri dish, sufficient nutrient broth added to make a mud of it, and this was then spread out with a Klein's platinum spreader, and, after five days' incubation at 37° C., examined for growth. I found that this method gave just as constant results as the former (a), and it had the advantage of saving one incubation and the corresponding number of days in time.

Result.—*M. melitensis* was recovered in this manner for 16 days.

Remark.—This experiment and the last are quite comparable in that the *M. melitensis* culture used was not only the same, but that it was kept dry with an inorganic environment free from organic matter, and

the duration of reproductive vitality was much the same; on cover slips 15 days, in sand 16 days.

2. *In Various Malta Soils.*—In preliminary experimentation on the sterilisation of these, they were all found after sterilisation by dry heat to be excessively alkaline, a condition seriously prejudicial to the vitality of *M. melitensis*. This was found to be due to the large amount of calcium carbonate they contained, some of which, by the dry heat used, was converted into calcium oxide, which on the addition of water became calcium hydrate. This caused one to examine the reaction of the various soils as received, by thoroughly stirring up and shaking each, and then shaking a little in distilled water in a test-tube and taking the reaction of that. All the specimens were thus found to be slightly alkaline to begin with. So sterilisation was now done by putting the specimen of soil in a beaker, half filling this with distilled water and placing it in the autoclave at 115° for 30 minutes; this was found to effect sterilisation without any alteration of alkalinity.

(a) *In Greyish Yellow Soil, with additional Organic Matter.*

This soil was personally obtained from a field in Sliema, was sterilised as just described, sterility verified by broth culture, a portion in bulk equal to about 10 c.c. placed in a sterile test-tube and put in incubator at 37° C. till dry, then on to it was poured, by means of a sterile pipette, a broth growth of *M. melitensis* (still Second Generation from Human Spleen, Bowles), till its upper $\frac{1}{3}$ was quite saturated with moisture, then tube was plugged with sterile wool and placed in laboratory cupboard. Twice in each week two portions from the upper surface were planted out in the manner described in K 1 for sand, incubated, and examined for growth. It was noticed, as the experiment progressed, that the upper surface of the soil got apparently dry, but that at the bottom of the test-tube it became damp from percolation of the nutrient broth downwards, and this dampness persisted till the conclusion of the experiment; so that the *M. melitensis* present must have been continually in the presence of water vapour. As in K 1, it was found that planting out on agar, as described, gave just as constant results as planting out in broth and then sub-culturing from this.

Result.—*M. melitensis* was thus recovered from this soil for 91 days.

(b) *In Reddish Soil without additional Organic Matter.*

This soil was also personally obtained from a Sliema field; it was very similar in composition to that used in the preceding experiment, differing mainly by containing a little under 1 per cent. of iron oxide, to which its reddish colour was due. It was sterilised under water in the autoclave at 115° C., sterility verified by broth culture, and sufficient placed in a small sterile Petri dish (3 cm. in diameter) to give a depth of about $\frac{3}{16}$ to $\frac{1}{4}$

of an inch. An emulsion of same quantity and brand of *M. melitensis* as in Experiment K 1 was made in 5 c.c. of distilled sterilised water, and, by means of a sterile pipette, was distributed all over the surface of the sterilised soil contained in the Petri dish. Two small portions of this were planted out in agar and in broth twice weekly in the manner already described, incubated and examined for growth.

Result.—*M. melitensis* was thus recovered from this soil for 80 days.

(c) *In White Soil without additional Organic Matter.*

This specimen of soil was obtained from an area of land where building operations were going on, and consisted very largely of the *débris* from the stone cutting, shaping, and smoothing operations which, as is usual in Malta, were carried out on the spot where the finished stone was wanted for use. It is a very soft friable Globigerina limestone, and the *débris* used contained only a mere trace of organic matter. With this the experiment just described was exactly repeated in every particular.

Result.—*M. melitensis* was recovered for 24 days.

(d) *In Recently well-manured Soil, Sterilised and kept Wet.*

This experiment was intended to contrast with the latter in the amount of organic matter present in the soil, it being very great in this experiment, very little indeed in the last one, and also in the amount of water present, a similar difference being maintained. Recently (five weeks) manured soil was obtained from the Argotti Botanical Gardens, dried, pulverised in a mortar, sterilised as in the preceding three experiments, an amount equal in bulk to about 10 c.c. put in a sterile test-tube and well shaken down; and then on to it was poured, by means of a sterile pipette, an emulsion of *M. melitensis* grown on agar made from the same quantity, brand, and generation of *M. melitensis* as in the preceding experiments, in 5 c.c. of distilled sterilised water. This soil was further kept saturated with moisture by dropping on it from time to time sterilised tap water from a sterile pipette, and was kept at laboratory temperature (15° C.) for the whole period of the experiment. From it two portions were planted out in broth, and on agar twice weekly in the way described in K 1, then incubated and examined for growth. The experiment was started on December 14, 1904, and each planting out yielded *M. melitensis*, which, as usual, was duly verified. During the examination and verification of the growth of January 8, it was noticed that the resulting colonies, while resembling the usual *M. melitensis* colony in every other particular, had less sharply defined, less abrupt margins, and that the microscopical preparations contained a few bacillary forms. By February 12 the new colonies of that date presented slightly crenated edges, shading away on the agar, though quite similar in size and shape

to standard *M. melitensis* colonies of same duration of growth on same agar, and now consisted almost entirely under the microscope of small bacillary forms, when stained, of about $\frac{2}{3}$ the diameter of a normal *M. melitensis*, and three times its length. A sub-culture of this was now put through *all* the tests specified in Part I of these Reports, for the recognition and verification of *M. melitensis*, behaving in all particulars like standard *M. melitensis* save in the morphological details mentioned. These bacilli in hanging-drop preparations were feebly motile. Many specimens were stained for flagellæ according to Rossi's method, but none were demonstrated. It will be remembered that Gordon, in a paper in the *Lancet*, March 11, 1899, described flagellæ in connection with *M. melitensis*. I have not succeeded in verifying this. Successive sub-cultures of this growth of February 12, for 10 generations, in broth and on agar were now made, but there was no reversion to the coccil form; the Tenth Generation was exactly like the First; but a sub-culture from the Ninth Generation into peptone water (made for the purpose of ascertaining the presence or absence of indol and nitrite formation, neither present), showed in a stained specimen both cocci and bacilli, and intermediary forms such as French bacteriologists speak of as "cocco-bacille." On February 22 a rabbit which had never been experimented upon, and whose blood gave no trace of agglutination with standard *M. melitensis*, was injected intra-cerebrally with the usual aseptic precaution with $\frac{1}{2}$ c.c. of an emulsion made from this same Ninth Generation. Its temperature rose to 105° F. the same evening and 106° F. the following evening, after which it fell to normal and never rose again. On February 25 there was a distinct agglutination reaction on *M. melitensis*, with its blood serum in a dilution of $\frac{1}{32}$; this had increased on March 5 to a dilution of $\frac{1}{360}$. On February 28 1 c.c. of blood was taken aseptically from the animal's left internal saphenous vein, placed in 19 c.c. of nutrient broth, and incubated in the usual way. There being absolutely no trace of growth obtainable from this up to March 10, the animal, which had fully recovered from the operation, was that day chloroformed, a *post-mortem* made, and inoculations made into both broth and agar from the brain, heart's blood, urine, spleen, liver, and kidneys; no growth was obtained from any of these.

Concurrently with all this, the periodical plantings out had been carried on, but no growth was obtained from any planting out later than March 7, though these were continued till March 22.

Remarks.—A bacillary form in pure cultures of *M. melitensis* has been noticed by various workers, but not apparently in so marked a degree as in this instance. As an intra-cerebral injection of the coccil form of *M. melitensis* usually produces in a rabbit death in four or five days with presence of *M. melitensis* in the various organs, the bacillary form produced, as described, is obviously of less virulence.

Result.—*M. melitensis* was recovered from this sterilised, manured, and saturated soil rich in organic matter for 83 days, a bacillary form of *M. melitensis* deficient in virulence being developed.

(e) *In Recently Manured Non-Sterilised Soil.*

The same soil as in the last experiment was used and treated in precisely the same way save that it was not sterilised.

As it was anticipated that very rapid overgrowing of the *M. melitensis* put in (the viability of which was as usual tested by a control) would take place, a little was planted out daily, a small portion being placed on the surface of agar in a Petri dish, made into mud with nutrient broth, and distributed by means of a Klein's platinum spreader all over the surface of agar in three successive Petri plates. Although in this manner discrete isolated colonies were obtained in the third plate, no *M. melitensis* was ever recovered, though four separate repetitions of the experiment were made.

Result.—*M. melitensis* speedily crowded out by the other organisms present.

Summary of Results obtained as to Vitality of M. Melitensis Outside the Body, the same Brand, Generation and Quantity of M. melitensis being used throughout (except in B 1).

	Days.
A. In mixed broth culture with—	
1. Laboratory contamination.....	7
2. " "	7
3. Organisms derived from sweat, skin and urine	2
4. " " " "	Nil
5 to 10. " " " " and breath; in each	1
B. In pure culture media with a reaction of + 10 acid—	
1. On agar slope (source of <i>M. melitensis</i> not noted)	276
2. In litmus milk (source of <i>M. melitensis</i> as in all other experiments)	144
3. In peptone broth (ditto)	173
C. In urine (persistent acidity of Mediterranean Fever urine as described)—	
1. In unsterilised normal healthy urine, four experiments	2, 5, 2 and 33
2. " " Malta Fever urine, six experiments	15, 24, 18 43, 36 and 49
3. " sterilised normal healthy urine, one experiment ...	17
4. " " Malta Fever urine, one experiment	33

	Days.
D. In diluted urine—	
1. Healthy urine diluted 100 times with sterile tap water	9
2. Mediterranean Fever (ditto)	79
E. In urine contaminated milk—	
1. Goat's milk contaminated with 1 per cent. healthy urine	3
2. " " 1 " Mediterranean Fever urine	38
F. In urine dried on fabrics—	
1. In Mediterranean Fever urine dried on lint	5
2. " " " navy serge ...	78
G. In sterilised tap water	50
H. In unsterilised tap water—	
1. Tank water	10
2. Potable water	72
I. In unsterile sea water, two experiments	11 and 46
J. Dry on cover slips	15
K. In various earths—	
1. In sand free from organic matter	16
2. „ various Malta soils—	
a. In sterilised grey-yellow soil with added organic matter.....	91
b. In sterilised reddish soil without added organic matter.....	80
c. In sterilised white soil almost free from organic matter.....	24
d. In sterilised well-manured soil rich in organic matter (bacillary forms of <i>M. melitensis</i> developed)	83
e. In non-sterilised well-manured soil	Not recovered

IV. ON THE RECOVERY OF *MICROCOCCUS MELITENSIS* FROM THE URINE OF MEDITERRANEAN FEVER PATIENTS.

By J. CRAWFORD KENNEDY, Captain R.A.M.C., Member Mediterranean Fever Commission, Malta, April, 1905.

Since September, 1904, this work has been more than trebled, and special attention has been paid to the quantities excreted and to the period of disease during which the excretion is greatest.

The following table is a summary of the work done :—

	No. of samples examined.	No. of times <i>M. melitensis</i> recovered.
September	347	6
October	217	6
November	581	63
December	398	43
January	201	19
February.....	110	19
March to April 2 ...	120	30
Total	1974	186

Percentage of recoveries..... 9½ per cent.

The number of cases examined was 61, and from 33 of these *M. melitensis* was recovered. Therefore *M. melitensis* was recovered from 54 per cent. of the cases examined. Deduct from this the cases which were examined less than 10 times—it leaves 50 cases and 31 recoveries, or 62 per cent. ; 43 cases were examined over 20 times, with 31 recoveries, or 72 per cent.

The method of examination was the same as described by Major Horrocks in a former report, and each recovery put through the usual tests.

In order that the work may be taken in at a glance, I have prepared a list of the cases from which *M. melitensis* was recovered, giving particulars of number of samples and recoveries, quantity and period of disease, also a chart of the temperature. In those cases which supplied many recoveries the whole chart is given, in those with only one recovery, only the previous and subsequent two or three days' temperature is given.

No.	Name.	No. of samples examined.	No. of times <i>M. melitensis</i> recovered.	Greatest No. of colonies <i>M. melitensis</i> found in a cubic centimetre urine.	No. of times <i>M. melitensis</i> recovered when temperature of previous or of subsequent 24 hours not above 99°.	Earliest and latest day of illness on which <i>M. melitensis</i> recovered.	Remarks.
1	Kinsella	97	27	189	16	21 111	See chart. This case examined all through illness. The excretion of <i>M. melitensis</i> was almost entirely during convalescence.
2	Bean	44	23	450	7	58 84	See chart. A normal temperature for 7 days during excretion of <i>M. melitensis</i> .
3	Ralph	76	18	440	2	89 133	See chart. Excretion of <i>M. melitensis</i> after 33 days' normal temperature.
4	Smith (Rifle)	64	6	309	—	74 114	See chart.
5	Gane	29	14	1068	7	108 145	See chart. Excretion after 27 days of practically normal temperature.
6	Charlton	35	4	18	4	82 102	See chart. Excretion after 30 days' normal temperature.
7	Bolt	151	23	129	—	74 165	See complete chart.
8	Anthony	91	3	Innumerable	—	81 156	See chart. Colonies so thick in one sample as to be uncountable—from 500 to 800 in one drop urine.
9	Surmin	93	48	Innumerable	27	174 249	See chart. Another case of a sudden gush of <i>M. melitensis</i> in urine.
10	Groom	(74 days) 70	(41 days) 1	3	1	83	See chart.
11	Mitchell	4	1	6	1	77	See chart. Excretion after 12 days' normal temperature.
12	Rivers	4	1	3	1	43	See chart.
13	Cannole	31	2	3	2	102 112	See chart. Excretion after 15 days' normal temperature.

List of Cases from which *M. melitensis* has been Recovered, giving particulars of Quantity and Period of Disease—*contd.*

No.	Name.	No. of samples examined.	No. of times <i>M. melitensis</i> recovered.	Greatest No. of colonies <i>M. melitensis</i> found in a cubic centimetre urine.	No. of times <i>M. melitensis</i> recovered when temperature of previous or of subsequent 24 hours not above 99°.	Earliest and latest day of illness on which <i>M. melitensis</i> recovered.	Remarks.
14	Campbell	71	2	6	—	93	See chart.
15	Walker	25	2	116	—	55	See chart. Excretion during convalescence.
16	Silburn	23	2	9	2	60	See chart.
17	Turner	80	1	3	—	52	See chart.
18	Bagwell	29	1	3	—	77	See chart.
19	Rentcome	40	1	4	1	99	See chart.
20	Marchant	55	1	15	—	72	See chart.
21	Donovan	46	1	3	1	57	See chart.
22	Bennett	41	1	4	1	83	Excretion after 24 days' normal temperature; quite convalescent.
Total		1199	183	Earliest day recovered		21	Out of 20 cases examined during convalescence 11 were found to be excreting <i>M. melitensis</i> .
Average...		—	15·3 per cent.	Latent day recovered		249	

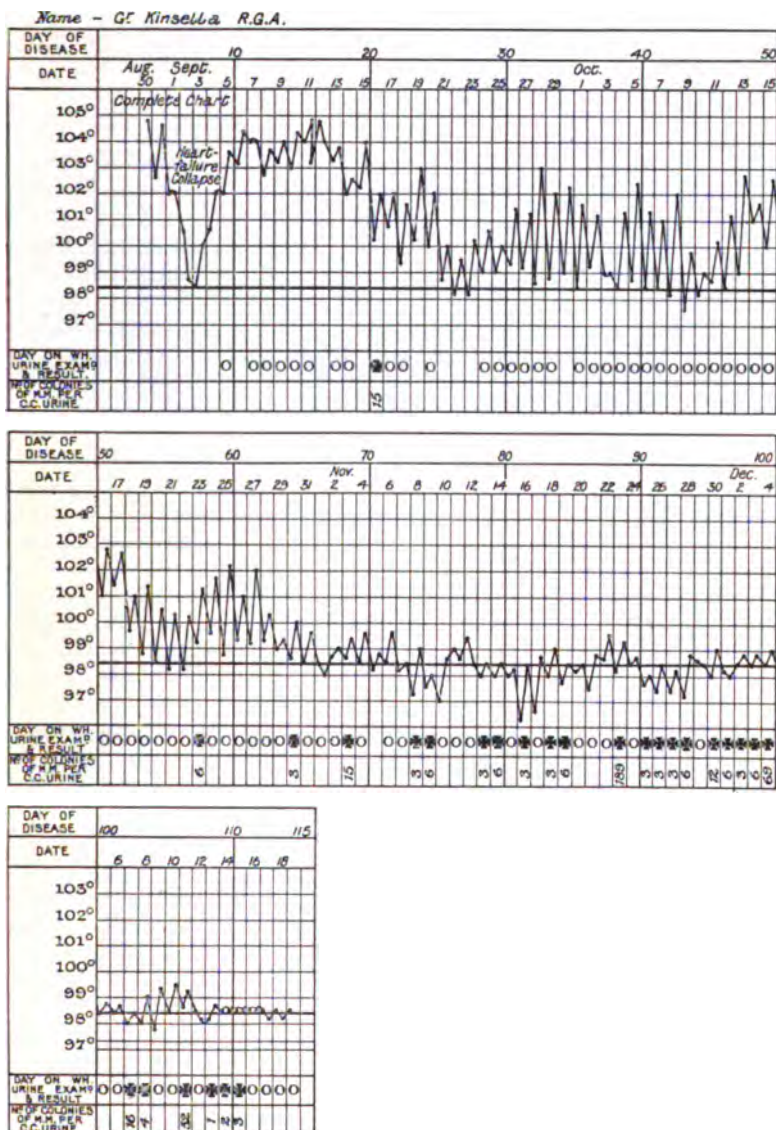


Chart 1.—KINSELLA.

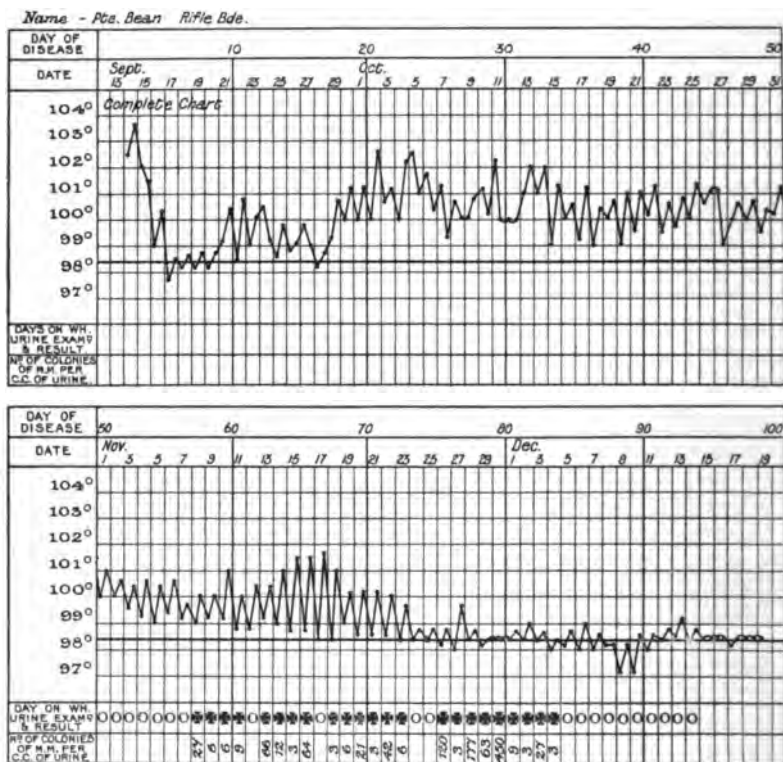


Chart 2.—BEAN.

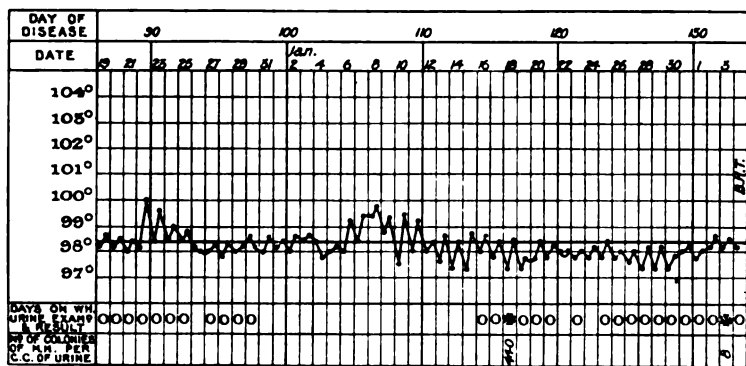
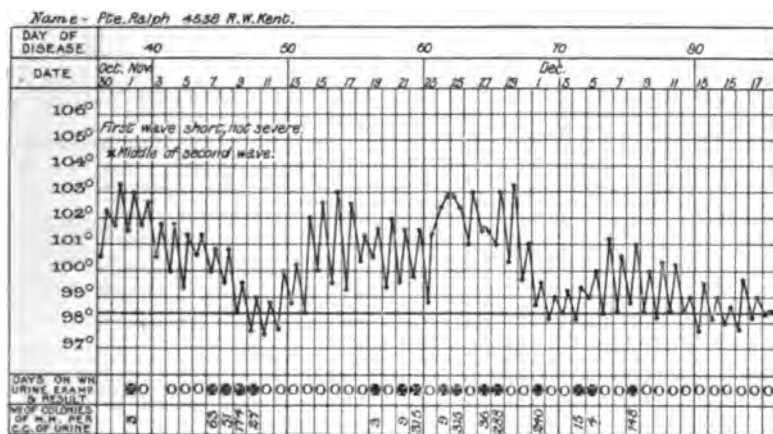


Chart 3.—RALPH.

62 Captain J. C. Kennedy. *On the Recovery of M. melitensis*

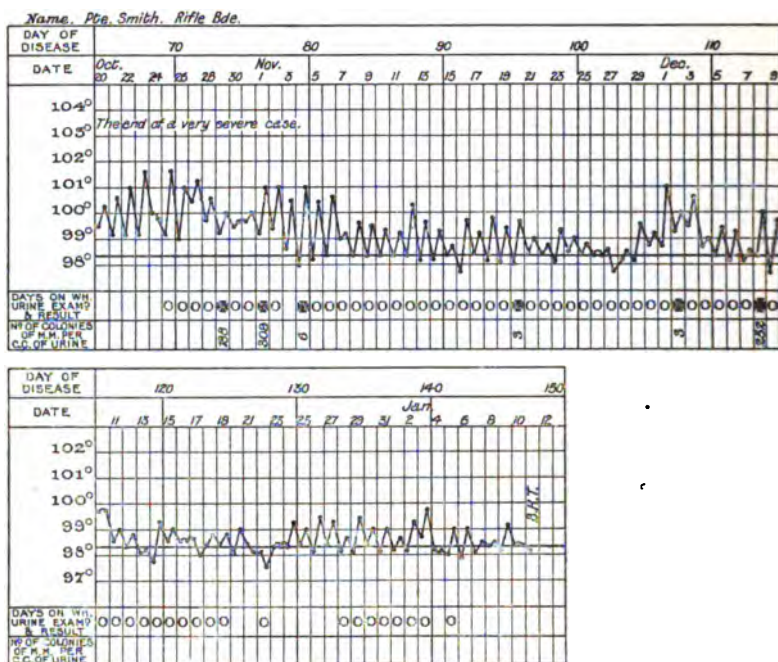


Chart 4.—SMITH.

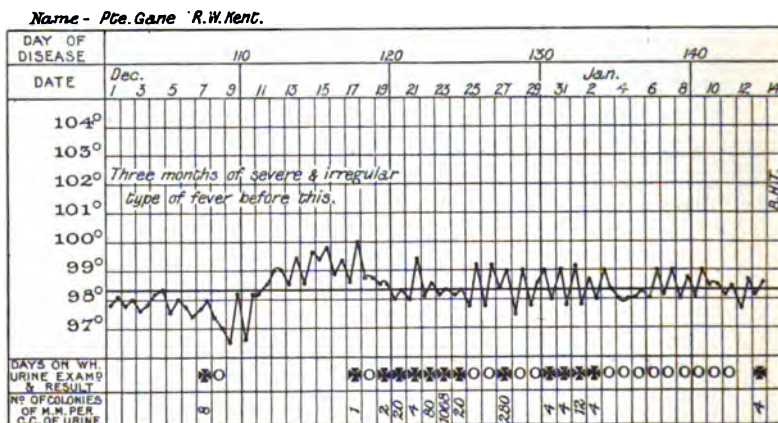


Chart 5.—GANE.

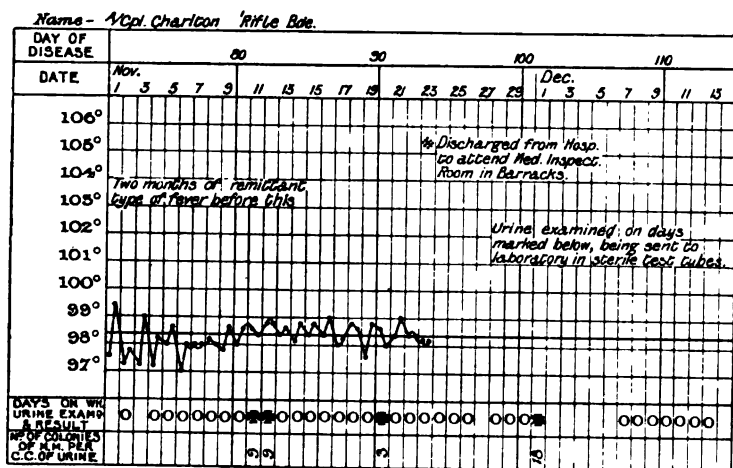


Chart 6.—CHARLTON.

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Name - Boy Bolt R.G.A.

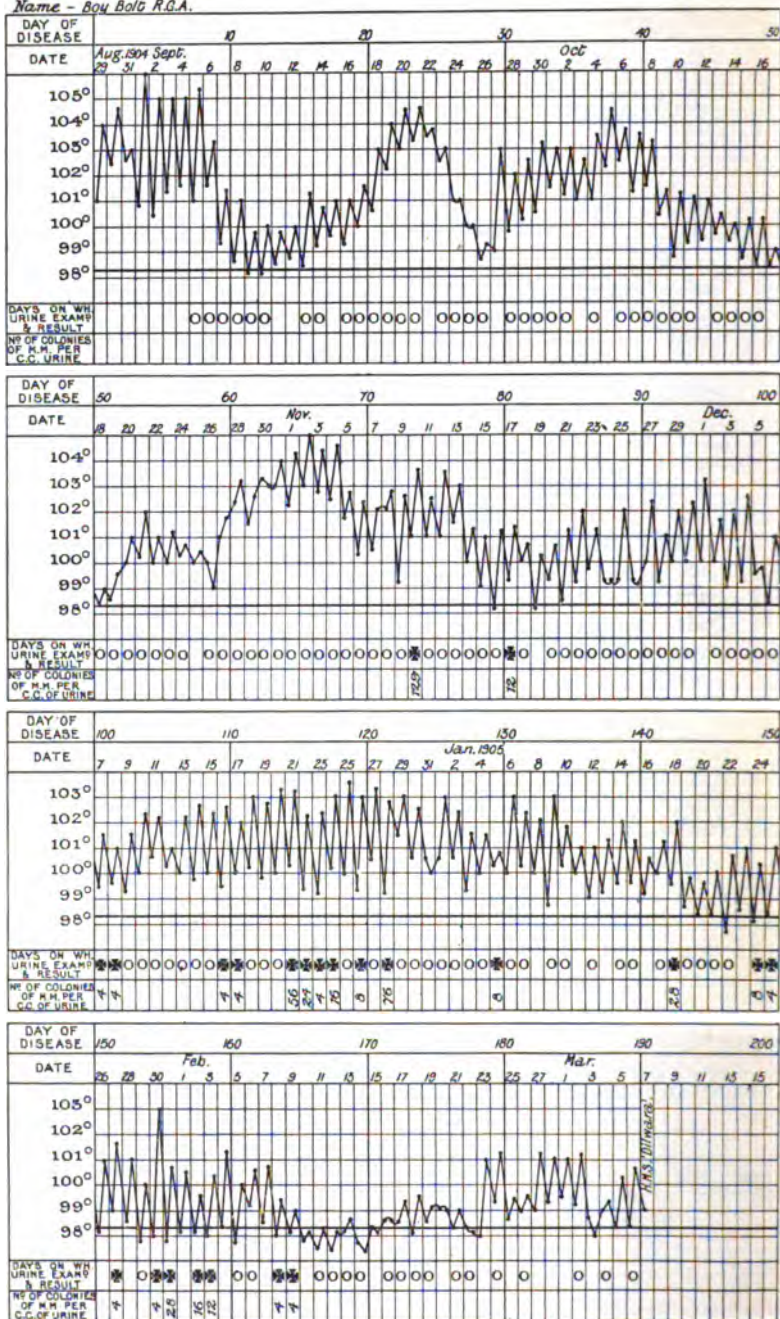
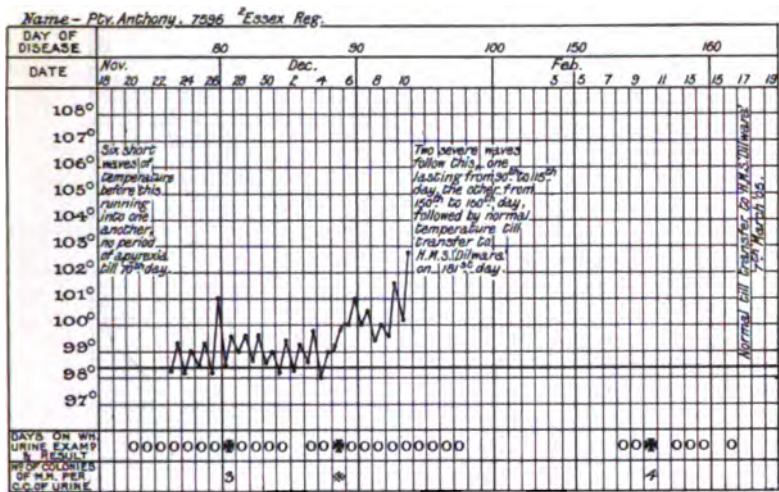
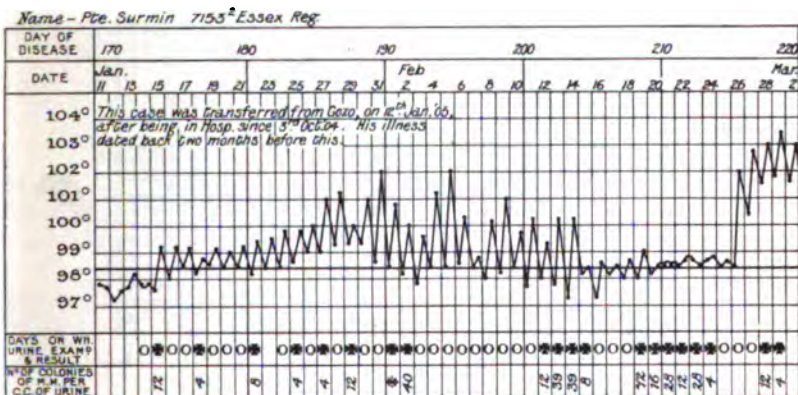


Chart 7.—BOLT.



* Colonies absolutely innumerable, any number between 200 to 500 in each drop of urine.

Chart 8.—ANTHONY.



* Colonies on plate were innumerable. Almost as thick as an artificial emulsion.

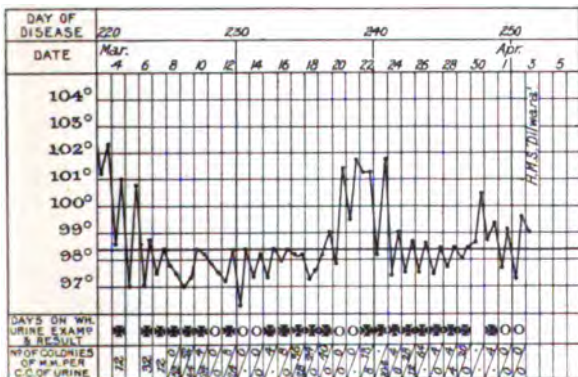


Chart 9.—SURMIN.

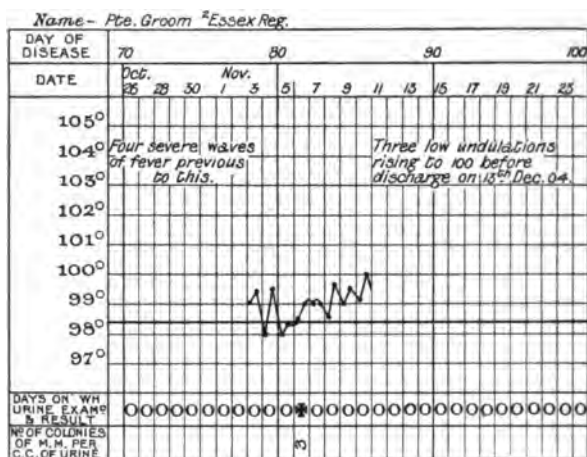


Chart 10.—GROOM.

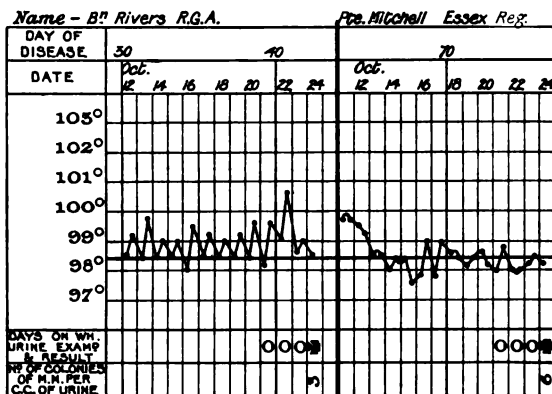


Chart 12.—RIVERS.

Chart 11.—MITCHELL.

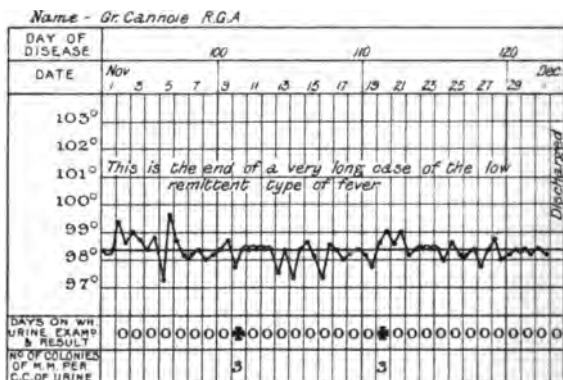


Chart 13.—CANNOLE.

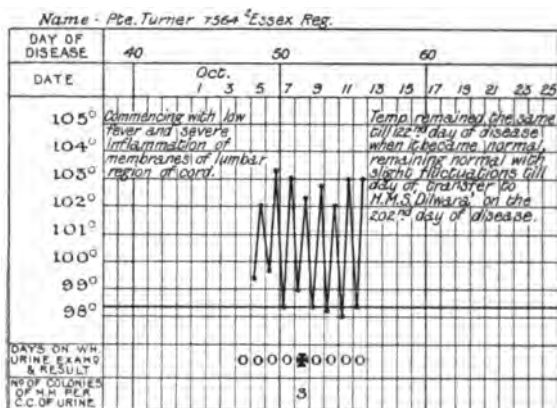


Chart 17.—TURNER.

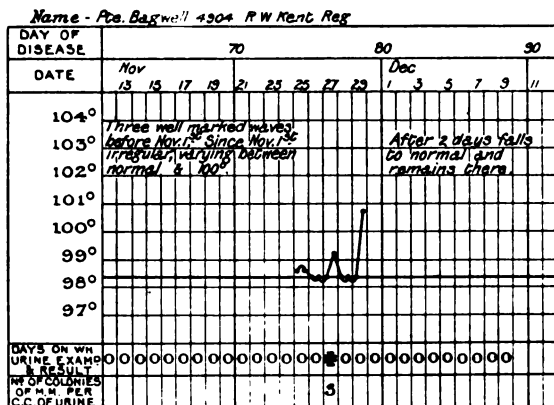


Chart 18.—BAGWELL.

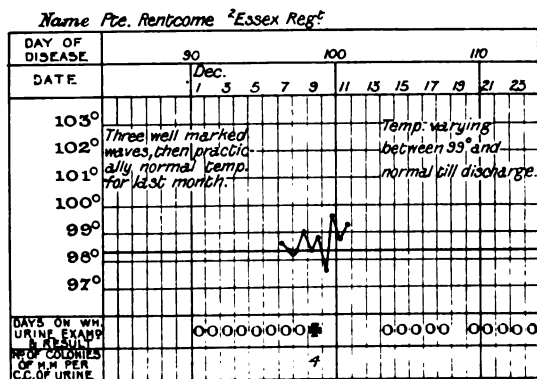


Chart 19.—RENTCOME.

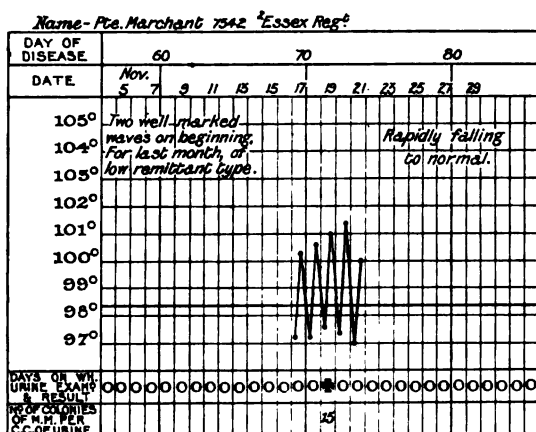


Chart 20.—MERCHANT.

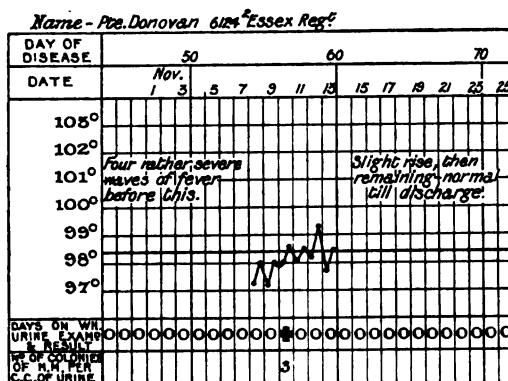


Chart 21.—DONOVAN.

Summary.—The excretion of *M. melitensis* in the urine would appear to be of two kinds:—

- (1) A sudden enormous gush which stops as suddenly as it appears.
- (2) A long continued excretion of small quantities.

As examples of the first see Cases Nos. 8 and 9.

As examples of the second see Cases Nos. 1, 2, 5, 7 and 9.

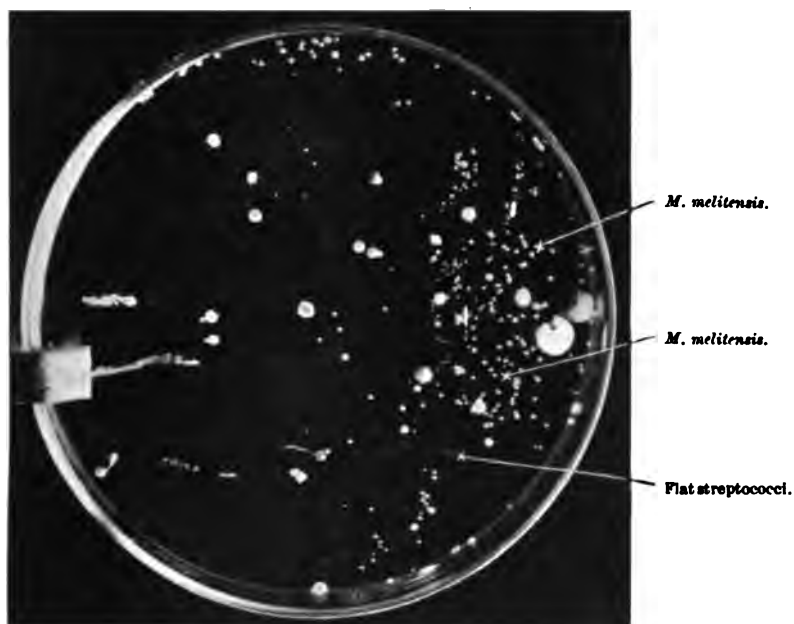
The period of disease most favoured is early convalescence or the last stages of the fever, especially just as a "wave" of fever is subsiding and the temperature reaching normal. It will be noticed that excretion tends to stop if another "wave" begins (see Charts 3, 8 and 9).

The time of day or rather the period of the 24 hours during which

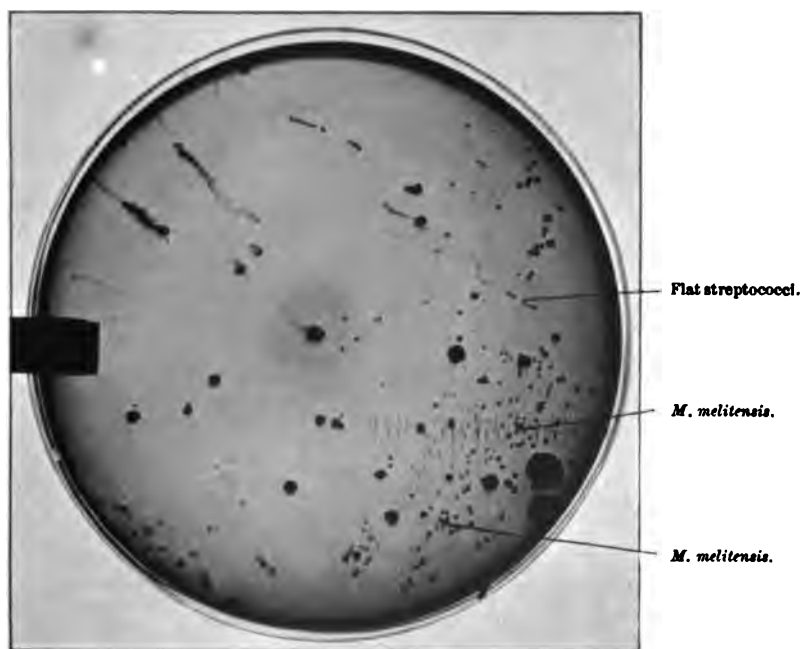
There is a well-defined relationship between δ and α in the Cu_2O system. The δ values for samples of the same composition are plotted in Figure 1. The δ values for the Cu_2O samples are in the range 0.0005–0.0015. On the basis of the δ values, samples are classified as below and above the $\delta = 0.001$ line. The δ values for the Cu_2O samples are in the range 0.0005–0.0015. The δ values for the Cu_2O samples are in the range 0.0005–0.0015.

the \mathcal{H}_∞ norm of the closed-loop system is bounded by γ . The \mathcal{H}_∞ norm of the closed-loop system is defined as the maximum singular value of the transfer function matrix $G(s)$ over the complex frequency s in the right half plane. The \mathcal{H}_∞ norm of the closed-loop system is bounded by γ if and only if the following two conditions are satisfied: (1) the closed-loop system is stable; (2) the \mathcal{H}_∞ norm of the closed-loop system is bounded by γ . The \mathcal{H}_∞ norm of the closed-loop system is bounded by γ if and only if the following two conditions are satisfied: (1) the closed-loop system is stable; (2) the \mathcal{H}_∞ norm of the closed-loop system is bounded by γ .

[illegible][illegible]



Reflected light.



Direct light (same plate).

V. ON THE VITALITY OF *MICROCOCCUS MELITENSIS*
IN URINE (in which it has been excreted), ON CLOTH,
IN DUST, STERILE TAP WATER, AND STERILE MILK.

(Being Experiments 1 to 6, suggested by the Sub-Committee.)

By J. CRAWFORD KENNEDY, Capt. R.A.M.C., Member Mediterranean
Fever Commission. Malta, April, 1905.

Experiments 1 and 2.

How long does the *M. melitensis* retain its vitality in urine?

The following procedure was adopted:—A batch of urines was collected every day in sterile test-tubes plugged with cotton wool, and after $\frac{1}{4}$ c.c. from each had been planted out on plates they were laid aside. The next day, and every day till the 4th day, $\frac{1}{4}$ c.c. was again planted out on Petrie dishes. On the 4th day the plates made on the 1st day were sufficiently incubated, and the presence or absence of *M. melitensis* was noted. Those urines from which *M. melitensis* was absent were then discarded.

It was soon found that *M. melitensis* could be recovered after four days in urine, so the urines were left undisturbed for the four days until it was determined which samples contained *M. melitensis*. Those samples were then plated out day after day, the more plates being used each day according to the length of time the urine had been kept.

As the majority of the samples contained *M. melitensis* in very small quantities, the urine was disturbed as little as possible, so that, supposing the *M. melitensis* had been found in one sample on the 5th day, the next sample of urine was not plated out till the 6th day, and so on. In this way 525 samples of urine were gone through. In 53 of these *M. melitensis* was recovered in the first instance, but in only 12 was it recovered a second time.

The following table (p. 72) shows at a glance the result of the examination of these 12 samples.

The *M. melitensis* has therefore been recovered from urine 16 days old. The points that favour its existence in urine are—

1. Acidity,
2. Absence of other organisms, whether acid or alkaline.

1. The urine of Mediterranean Fever patients is markedly acid, and if moderately free from contaminating germs will remain so for a

Date.	Name.	Number of colonies of <i>M. melitensis</i> found on 1st day per plate.	Result of examination of urine after standing the number of days indicated. + = <i>M. melitensis</i> recovered.																						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Dec. 7	Kinsella ...	4	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	Holt.....	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	Gane	5	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	Gane	1	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Bolt.....	14	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	Gane	70 { A B O		+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	Gane	1																							
Jan. 1	Gane	3																							
10	Walker ...	29 (alkaline)																							
13	Gane	1																							
Feb. 1	Surmin ...	—																							
13	Surmin ...	13																							

very long time. Both the urines in which *M. melitensis* was recovered on the 16th day were acid on that day. The urine of Gane remained acid 29 days.

Two other samples (Kinsella, December 7 and 11, 1904) were tested daily for acidity to litmus; the sample of December 7 began to turn alkaline on January 27, and the other on January 26—i.e., 51 and 46 days respectively. At the same time these samples, when planted out on plates, were found to be very filthy by the 5th or 6th day.

In only one sample was *M. melitensis* recovered, when the urine gave an alkaline reaction—viz., Walker sample, January 10. This urine was alkaline on the 1st day, and *M. melitensis* was recovered after it had stood for six days.

2. The presence of other organisms in the urine in any quantity is fatal to the recovery of *M. melitensis*. I am inclined to think that it may exist alongside the others, but that on nutrient media the excessive acidity or alkalinity produced by these rapid growing organisms prevents its development.

On several occasions I have found colonies of *M. melitensis* in a very acid plate hiding under the shelter (as it were) of a large alkali producing colony, where the acid is neutralised by the alkali.

Colonies that grow under these difficulties are always very tiny, do not have the amber colour by transmitted light, and in salt solution tend to remain in chains; the subcultures, however, are typical.

The sample of Surmin, February 1, was one of the two which contained *M. melitensis* in enormous quantities, and should have been an excellent sample for this experiment. *M. melitensis* was easily recovered from it on the 3rd day, but could not be isolated on the 6th, as the plate was overgrown with acid streptococci.

I attach a photograph (Plate 2) of the plates made from this sample on the 1st and on the 3rd day. It will be noticed that they are practically pure cultures of *M. melitensis*. The second plate is not so vigorous a growth as the first, the colonies tend to be smaller and less well defined, though their number is just as great as in the first. Both were incubated for four days. I have taken them in such a way that the light is reflected from the surface of the colonies, which thus stand out in relief.

Experiment with Artificially Infected Urine.

On December 16, 1904, the *M. melitensis* from the urine of a patient was added to the freshly passed urine of another, until the urine became cloudy. This was allowed to stand in a sterile test tube plugged with cotton-wool.

M. melitensis was easily recovered up to December 22 (six days). On the 23rd the growth of *M. melitensis* on the plate was beginning to get faint.

On the 24th the growth of *M. melitensis* was merely a faint blue haze along the edge of the track left by the drops of urine as they ran over the plate.

On the 26th *M. melitensis* could not be recovered as the plates were quite overgrown with acid streptococci. The urine remained acid for six days, after when the experiment was stopped.

Results.—The *M. melitensis* retains its vitality in urine (naturally infected) for 16 days, provided the urine remains acid and is fairly free from contaminating organisms.

It has been isolated from a urine (naturally infected) which had turned alkaline after six days.

In an artificially infected urine *M. melitensis* retained its vitality for seven days.

Experiments 3, 4, 5 and 6. Suggested by the Sub-Committee.

In carrying out these experiments, the great difficulty has been to know when one is dealing with a urine that contains the *M. melitensis*.

A urine collected one day and planted out on Petrie dishes has to be kept four days before the presence or absence of *M. melitensis* can be ascertained. By this time, even if *M. melitensis* be found in the plates, it is no guarantee that it has not died out by the 4th day, and in any case the vitality of any survivors has probably been seriously impaired.

To overcome this difficulty, I looked round for cases that would give a fairly regular supply of infected urine. In this I was fairly fortunate, and then my procedure was as follows:—

A sample of urine was collected every day from these patients, and every day it was—

1. Plated out on nutrose glucose litmus. This served as a control.
2. Allowed to dry on pieces of khaki drill.
3. Mixed with sterile dust.
4. Added to sterile tap water.
5. Added to sterile milk.

On the 3rd and 4th days the control plates were examined, and if *M. melitensis* was present the Experiments (2, 3, 4, 5) were proceeded with. This meant increasing the work greatly, as many samples proved useless.

The majority of samples treated in this way, and proved to be infected, contained *M. melitensis* in very small quantities—i.e., 4 to 30 colonies per cubic centimetre urine. It will be readily understood that the chances of recovering it again after diluting the sample 100 times are very small.

$$\begin{aligned} \frac{\partial \mathcal{L}}{\partial \mathbf{w}_1} &= \mathbf{w}_2 + \mathbf{w}_1 + \mathbf{w}_1 \mathbf{w}_2 + \mathbf{w}_1 \mathbf{w}_2 \mathbf{w}_1 + \mathbf{w}_1 \mathbf{w}_2 \mathbf{w}_1 \mathbf{w}_2 + \dots \\ &= \mathbf{w}_2 + \mathbf{w}_1 + \mathbf{w}_1 \mathbf{w}_2 + \mathbf{w}_1 \mathbf{w}_2 \mathbf{w}_1 + \mathbf{w}_1 \mathbf{w}_2 \mathbf{w}_1 \mathbf{w}_2 + \dots \end{aligned}$$

The authors would like to thank the referees for their constructive comments. The authors would also like to thank the editor for his/her constructive comments.

where $\mathbf{A} = \mathbf{A}(\mathbf{r})$ is the vector potential, \mathbf{r} is the position vector, and \mathbf{r}_0 is the position vector of the center of the sphere. The vector potential \mathbf{A} is given by

• *Staphylococcus aureus* (Staph aureus)

1. *Journal of the American Medical Association*, 1997; 277: 1033-1036.

[illegible][illegible]

It is not clear whether the authors are referring to the fact that the model is not a perfect representation of the real world, or to the fact that the model is not a perfect representation of the model itself. The authors are likely referring to the fact that the model is not a perfect representation of the real world, as the model is a simplification of the real world and therefore cannot capture all the details of the real world. The authors are likely referring to the fact that the model is not a perfect representation of the model itself, as the model is a simplification of the real world and therefore cannot capture all the details of the real world.

[illegible]

1. The first step is to identify the key components of the system. This involves understanding the hardware, software, and data involved in the process.

[illegible]

where \mathbf{w}_i is the weight vector of the i th hidden unit, \mathbf{w}_0 is the bias vector, $\mathbf{w}_0 = [w_0, w_1, \dots, w_n]$, $\mathbf{w}_i = [w_{i1}, w_{i2}, \dots, w_{in}]$, $\mathbf{x} = [x_1, x_2, \dots, x_n]$, $\mathbf{y} = [y_1, y_2, \dots, y_n]$, $\mathbf{z} = [z_1, z_2, \dots, z_n]$, $\mathbf{v} = [v_1, v_2, \dots, v_n]$, $\mathbf{u} = [u_1, u_2, \dots, u_n]$, $\mathbf{t} = [t_1, t_2, \dots, t_n]$, $\mathbf{p} = [p_1, p_2, \dots, p_n]$, $\mathbf{q} = [q_1, q_2, \dots, q_n]$, $\mathbf{r} = [r_1, r_2, \dots, r_n]$, $\mathbf{s} = [s_1, s_2, \dots, s_n]$, $\mathbf{v} = [v_1, v_2, \dots, v_n]$, $\mathbf{u} = [u_1, u_2, \dots, u_n]$, $\mathbf{t} = [t_1, t_2, \dots, t_n]$, $\mathbf{p} = [p_1, p_2, \dots, p_n]$, $\mathbf{q} = [q_1, q_2, \dots, q_n]$, $\mathbf{r} = [r_1, r_2, \dots, r_n]$, $\mathbf{s} = [s_1, s_2, \dots, s_n]$.

It is important to note that the above results are based on the assumption that the distribution of the error term is normal. If the error term is non-normal, the results may be biased. However, the normality assumption is reasonable in this context, as the dependent variable is a continuous variable and the error term is expected to be normally distributed.



Urine, Surmin, February 1.

1. $\frac{1}{4}$ c.c. plated out on Petrie dish (nutrose glucose-litmus-agar) on February 1, incubated four days.
2. $\frac{1}{4}$ c.c. plated out on February 4 and incubated four days.

I.

Experiment 4.

How long does *M. melitensis* in urine, when dried on cloth, retain its vitality?

A. A series of experiments was first made with urine artificially infected by *M. melitensis*. The method adopted was as follows:—

The cloth used was khaki drill, thoroughly sterilised and cut up into small pieces of $\frac{1}{2}$ inch square. One-quarter of a cubic centimetre of the infected urine was then placed on each piece of cloth, and allowed to dry naturally. At varying intervals a piece was teased out in sterilised water, and the water planted out on a series of Petrie dishes containing the nutrient medium. This was found to be the best way of recovering the *M. melitensis*, as, if the urine-contaminated cloth was first treated in broth, the rapid-growing organisms would render its recovery impossible.

(1) January 8. *M. melitensis* recovered from urine added to Mediterranean Fever urine 24 hours old with acid reaction. Procedure as above, except that the cloths were put in the incubator for six hours at 37° C. to dry the quicker.

Series of plates were made on January 10, 12, 14, and 16 from pieces of the cloth. *M. melitensis* was not recovered on any of these days; the plates were found to be very acid, and overgrown.

On the 17th another piece was teased out in sterile distilled water, and phenol phthalein added as an indicator. It required two drops of $\pi/10$ alkaline solution to render the mixture alkaline to the indicator. Two more drops of the alkaline solution were added, and then the mixture plated out on a series of Petrie dishes. The result was that *one colony* of *M. melitensis* was found in one of the plates. This colony was put through the tests, and proved to be *M. melitensis*. This experiment was repeated with another cloth on January 23, but without success.

Result:—*M. melitensis* found living in a very filthy cloth after nine days.

(2) January 16. An experiment similar to the preceding was started, with this difference that the urine was fresh and it was allowed to dry naturally. A control plate was made from the infected urine, and the cloth examined on January 17, 18, and 23. The result in every case was nil, the plates all being very foul and acid.

(3) January 20. Another urine was artificially infected and the same procedure carried out. A control was made from the urine on the 1st day and *M. melitensis* was easily recovered.

The cloth was examined on the 21st and 25th and *M. melitensis* was easily recovered on these days.

In contrast to the other two experiments the plates were very clean and comparatively free from contaminations.

The cloth of the 21st provided *M. melitensis* in great quantities, that of the 25th rather scantily.

On February 1 another cloth was examined. No *M. melitensis* was recovered, the plates were very clean.

On February 3 the cloth gave a few colonies of *M. melitensis*, this was the 14th day of the experiment. I continued examining these cloths every other day but never found *M. melitensis* again; the plates were always very clean, so that there was no chance of its being hidden by other organisms as was the case in the two former experiments.

Result.—*M. melitensis* found alive after 14 days.

B. The carrying out of this experiment with a naturally infected urine was very difficult and disheartening; it was only after many failures that I obtained a suitable urine.

This was urine of Surmin, February 1; it was not one of the samples which I had been using in the series of cloth experiments and so had not been put on to cloth on the 1st day. But having found *M. melitensis* in great quantities after the plate had been incubating three days and having fortunately kept the sample, I was able to put it on cloth when it was three days old; at the same time I made a control plate from this urine, and by looking to the report dealing with the vitality of *M. melitensis* in the urine, the photographs of this control plate and the plate of the 1st day will be seen side by side, showing the presence of *M. melitensis* in enormous quantities.

February 4. Urine of Surmin, February 1, put on cloth. Control as above.

February 7. Cloth examined. *M. melitensis* recovered in great quantities.

February 17. Cloth examined. No *M. melitensis* recovered.

February 21. Cloth examined. One colony of *M. melitensis* recovered. This colony was of a very dark amber colour. It answered all the tests for *M. melitensis* perfectly. I continued for some days making plates from the cloth but got no further recovery, though everything was favourable, as the plates showed very little growth.

Result.—*M. melitensis* excreted in urine dried on cloth will retain its vitality for 17 days, though it tends to die out before the 13th day.

It should be remembered that this sample of urine had stood for three days before being put on cloth, so that the vitality of the *M. melitensis* had probably been impaired.

II.

Experiment 3.

How long does *M. melitensis* retain its vitality in dust moistened with infected urine?

Procedure.—Dust used was the dust and mud from the road, which,

when dry, blows about as a very fine powder. It was thoroughly sterilised in hot air chamber. The infected urine was mixed up with it until it became of a pasty consistency. This was allowed to dry at room temperature and when dry was examined for *M. melitensis*. A small quantity of the dust was mixed up with sterile distilled water, thoroughly shaken, and the fluid pipetted off and planted out on plates. The dust generally was so fine that it would form an emulsion in the water and the whole mixture could be planted out. In this way it was found that *M. melitensis* could very readily be recovered.

A. A series of experiments was first performed with dust and urine which had been artificially infected with *M. melitensis* recovered from urine. The first two of these were unsuccessful owing to the very filthy state of the urine used. The third gave the following result:—

January 20. *M. melitensis* recovered from urine added to Mediterranean Fever urine; resulting emulsion added to dust which was allowed to dry at 16° C.

January 21. Small quantity plated out. *M. melitensis* in great quantities recovered on 3rd day, still more on the 4th.

January 25. Small quantity planted on one plate; same result as on 21st. *M. melitensis* recovered.

February 3. Another plate made. *M. melitensis* recovered in good quantities.

February 9 (20th day). Three plates made. Two contained *M. melitensis* in good quantity; one contained none. All the plates were very clean.

February 17 (28th day). Two plates made. *M. melitensis* recovered from both in great quantity.

February 25 (36 days). Two plates made. No *M. melitensis* was recovered from these plates.

March 1 (41 days). Six plates were made. *M. melitensis* was recovered from only two plates and in very small numbers.

March 4 (44 days). Seven plates made. Two of these each contained one colony of *M. melitensis*. This finished the supply of infected dust.

Result.—It is evident from this that the *M. melitensis* retained its vitality in the dust with no difficulty for one month, but after that time it died out quickly, but could be found when large quantities of dust were used and many plates made up to the 44th day.

B. Series of experiments were then made with urines naturally infected on the lines laid down in the beginning of this Report, viz., mixing consecutive series of fresh samples of urine with dust and then waiting until the controls were positive or negative for the presence of *M. melitensis*.

(1) In the first series *M. melitensis* was found in five controls, but only in quantities of 1 to 8 colonies per $\frac{1}{4}$ c.c. These dusts were plated out frequently and in large quantities, but no recovery of *M. melitensis* was made.

(2) In the second series 12 controls contained *M. melitensis*, but no *M. melitensis* was recovered from the dust. The number of colonies of *M. melitensis* in the controls of this series varied from 1 to 13 per $\frac{1}{4}$ c.c.

(3) On February 4 it was found that a sample of Surmin's urine passed on February 1 contained *M. melitensis* in large quantities (see Report on Vitality in Urine and Cloth Experiments), and some of it was mixed with dust and allowed to dry at room temperature.

February 5. Dust was dry and some was planted out on three plates. *M. melitensis* was recovered from each of these plates in great quantities, first appearing on the 3rd day.

Judging from the experiment with dust and artificially prepared urine, I allowed this dust to stand undisturbed till February 17 (13th day of experiment). On this day I again planted out some of the dust, but could not recover *M. melitensis*. I again planted it out on February 21, 25, and March 1, but could not recover it again.

Result.—Dust contaminated with naturally infected urine contained great quantities of *M. melitensis* after 24 hours, but after 13 days the *M. melitensis* had completely died out.

Note.—The same remark applies here as in the cloth experiment, viz., that the urine was three days old before being put in dust. Consequently, the vitality of *M. melitensis* may have been considerably impaired.

Experiments with Unsterilised Soil.

A red soil obtained from a garden bed was used. The method adopted was the same as for sterilised dust, but the proportion of sterile water to soil was greater. The urines used were the same as used in the second series of dust experiments. Plates were obtained in which the colonies were fairly discrete, but no *M. melitensis* was recovered.

III.

Experiment 5.

To determine vitality of *M. melitensis* in infected urine when added to sterile tap water. The method of procedure was as indicated above.

Fresh samples of urine were added to the sterile water contained in flasks of 100 c.c., or in test-tubes of 10 c.c.

One c.c. of urine was added to 100 c.c. and $\frac{1}{4}$ c.c. to 10 c.c. Controls were at the same time taken from each sample of urine and if *M. melitensis* was found the experiment was proceeded with.

In this way 20 samples of urine were tried, five in flasks and 15 in test-tubes.

Of those put in flasks three were found to contain *M. melitensis* in small quantities (viz., 72, 8, and 4 colonies per cubic centimetre). These three flasks were plated out on 4th, 7th, 8th, 14th, 15th, 17th, and 25th days. *M. melitensis* was not recovered.

Of those put in test-tubes, two were found to contain *M. melitensis* (4 and 12 per cubic centimetre). These were plated out on the 5th, 8th, 12th, and 15th days. *M. melitensis* was not recovered.

Being so unsuccessful with the experiment conducted with naturally infected urine, I then carried it out with urine artificially infected with *M. melitensis*.

February 14. The urine used was that of a Mediterranean Fever patient, freshly drawn, and the *M. melitensis* used had also been isolated from the urine of a Mediterranean Fever case.

The *M. melitensis* was added to the urine until a milkiness was visible. One c.c. of this emulsion was added to a flask containing 100 c.c. of sterile tap water and the flasks allowed to stand in the laboratory cupboard, where there was practically a constant temperature of 15° C.

On the following days, $\frac{1}{2}$ c.c. of the sediment was plated out on Petries containing nutrose-glucose-litmus-agar, February 15, 16, 17, 18, 19, 21, 23 and every day to March 4, 6, 11, 15. *M. melitensis* was recovered up to and on March 6, being the 20th day of the experiment.

For the first four days only one plate was taken from the flask, and the result was as follows:—On February 15 (the first completed day) the *M. melitensis* was recovered in great quantity, the first appearance being noticed on the 17th (48 hours' growth); on February 16 only five colonies appeared; on February 17 one only, and on the 18th none. Thereupon three plates were made each day and *M. melitensis* was always recovered from one or two of them.

The plates taken from February 21 to March 11 all contained numbers of colonies which were practically identical with *M. melitensis* to the naked eye by transmitted light, but by reflected light were a dull white and opaque instead of having a greenish blue halo appearance. This organism was a coccus slightly larger than *M. melitensis*, and which tended to remain in chains of four and five when emulsified in salt solution.

This organism gave a great deal of bother, especially towards the end of the experiment, as I found it increasingly difficult to separate the *M. melitensis* from it. When fishing a *M. melitensis* colony from the plate, and feeling certain that nothing else had been touched, I found that the sub-culture was more frequently than not contaminated by this organism, which in the sub-culture on nutrose-glucose-litmus grew with the production of acid. It was generally necessary to sub-culture twice before obtaining a pure growth of *M. melitensis*.

This organism when sub-cultured was an acid producing streptococcus not agglutinated by Mediterranean Fever serum and partly losing its stain when treated by Gram's method.

On March 11 and 15 no *M. melitensis* was recovered and the plates made on the latter date were very dirty, being overgrown with rapid growing alkaline colonies.

Conclusions. That *M. melitensis* tends to die out quickly in sterile tap water, but can be recovered from it up to the 20th day.

Experiment 6.

To determine survival of *M. melitensis* in infected urine which has been added to sterile milk. This experiment was carried out on the same lines as the former. In every case test-tubes containing 10 c.c. of sterile milk, to which a little litmus had been added, were used; the amount of urine added to each test-tube of milk was $\frac{1}{2}$ c.c.

December 12. Six samples of urine added to six litmus milk tubes. Control: no *M. melitensis* found in any one of the samples.

December 13. Five samples urine added to milk. Control: one sample (Kinsella) contained one colony *M. melitensis* per cubic centimetre. Four $\frac{1}{2}$ c.c. of the mixture of milk and this infected sample were plated out on four nutrose-glucose-litmus-agar plates on December 18, 24, 27. No *M. melitensis* was recovered.

December 14. Six samples urine added to milk. Control: one sample (Kinsella) contained two colonies *M. melitensis* per cubic centimetre. The infected milk was plated out on December 21, 24, and 27. No *M. melitensis* recovered.

December 19. Three samples urine added to milk. Control: none contained *M. melitensis*.

December 20. Ditto. Five samples of urine.

December 21. One sample urine from Boy Bolt. Control found to contain 56 colonies *M. melitensis* per cubic centimetre. The milk was plated out on the following days: December 27 (6th day of experiment). Milk showed no coagulation and remained alkaline. *M. melitensis* recovered.

December 31 (10th day of experiment). Milk still alkaline, no coagulation. *M. melitensis* recovered.

January 4 (14th day) }
January 8 (18th day) } Milk alkaline, *M. melitensis* not recovered.

January 12 (22nd day). Milk turned acid, *M. melitensis* not recovered.

December 24. Five samples of urine added to milk. Control: Bolt found to contain 16, and Gane 20 colonies *M. melitensis* per cubic centimetre. Bolt had turned the milk acid on the 31st. *M. melitensis* not recovered. Gane remained alkaline till January 4, *M. melitensis* not recovered.

December 24. Four samples urine added to milk. Control: one sample (Gane) was found to contain 280 colonies *M. melitensis* per cubic centimetre. The milk was plated out on the following days:

December 31 (4th day)	} Milk remained alkaline all this time. <i>M. melitensis</i> was recovered on each of these days.
January 4 (8th ")	
" 8 (12th ")	
" 12 (16th ")	
January 16 (20th day)	} By the 20th day (January 16) milk had turned acid. No <i>M. melitensis</i> was recovered after this.
" 20 (24th ")	
" 24 (28th ")	

After having recovered the *M. melitensis* up to the 16th day, it was decided to carry on the experiment without touching the infected milk till the 17th day of the experiment.

January 21. One sample of urine added to milk. Control gave no *M. melitensis*.

January 22. One sample of urine, ditto.

January 24. One sample urine added to milk. Control contained eight colonies *M. melitensis* per cubic centimetre. Milk plated on following days:—

February 10 (17th day of experiment)	} Milk remained alkaline. <i>M. melitensis</i> not recovered.
" 14 (21st " ")	
" 18 (25th " ")	

M. melitensis not recovered.

January 25. One sample of urine added to milk. Control contains four colonies per cubic centimetre. Plated out February 12 and 18. *M. melitensis* not recovered.

January 27. Same as January 25. Plated out on February 14. *M. melitensis* not recovered.

January 29. One sample urine. Control contained no *M. melitensis*.

January 30. Same as January 25. Plated out February 16 and 22. No *M. melitensis* recovered.

Six more samples were added to milk in the same way on February 4, 5, 6, and 7, but the controls were negative.

The *M. melitensis* recovered above was proved by the usual tests.

The only thing to be noted as regards cultural or other appearance is that in plates of the 12th and 16th days the colonies were of a darker amber colour than usual.

Summary.

Forty-eight samples of urine were added to milk; control experiments proved the presence of *M. melitensis* in 10 of them. *M. melitensis* was recovered from two of these; in one up to the 10th day and in the other up to the 16th day. In both cases the milk remained alkaline as long as the *M. melitensis* was recovered.

These urines must have been very clean and comparatively free from acid organisms. In most cases the milk became curdled in four days' time.

Conclusions.—*M. melitensis* will live in sterile milk which has been contaminated by infected urine as long as the milk remains alkaline or neutral to litmus.

Summary.

M. melitensis in the condition in which it is excreted in urine will retain its vitality—

1. Dried on cloth for 17 days.
2. In dust: for less than 13 days.
3. In sterile tap water. Not recovered.
4. In sterile milk for 16 days.

M. melitensis derived from urine, grown on media and then added to Mediterranean Fever urine, retains its vitality—

1. Dried on cloth for 14 days.
 2. Dried in dust for 44 days.
 3. Mixed with sterile tap water for 20 days.
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VI. A PRELIMINARY NOTE ON THE EXAMINATION OF THE BLOOD OF GOATS SUFFERING FROM MEDITERRANEAN FEVER.

By Dr. T. ZAMMIT, Member of the Mediterranean Fever Commission.

On June 14, as detailed by Major Horrocks, I examined the blood of six goats, which were brought to the lazaretto on June 12, and obtained the following results :—

Goat No. 1.—Strong immediate reaction, in dilution of 1 to 20.

Goat No. 2.—Strong immediate reaction, in dilution of 1 to 20.

Goat No. 3.—Strong reaction, after half-an-hour.

Goat No. 4.—No reaction.

Goat No. 5.—Strong reaction, after half-an-hour.

Goat No. 6.—Strong immediate reaction.

On June 15 the bloods were again examined, with identical results.

On June 18 about 5 c.c. of blood were taken from Goat No. 6, and distributed in six broth-tubes. On June 25 passages from the broth-tubes were made on to agar slopes, and the *M. melitensis* recovered in pure culture. This micro-organism was also recovered from the blood of Goat No. 5.

Blood has also been taken from Goats Nos. 1, 2, and 3, and distributed in broth-tubes as usual, but, so far, the *M. melitensis* has not been recovered.

Material from Abattoir.—Dr. Caruana Scicluna having suggested that possibly infected goats might be met with in the abattoir, I have examined 46 spleens removed with aseptic precautions, but, so far, have only recovered the *M. melitensis* from one. The blood from the goats was examined for agglutination with the *M. melitensis*, and a definite reaction was obtained in seven.

(For further details, see 'Proceedings of the Royal Society,' Series B, vol. 76, 1905, No. B 510.)

VII. PRELIMINARY NOTE ON GOATS AS A MEANS OF PROPAGATION OF MEDITERRANEAN FEVER.

By Major W. H. HORROCKS, R.A.M.C., Member of the Mediterranean Fever Commission.

(Reprinted from the 'Proceedings of the Royal Society,' Series B, vol. 76, 1905, No. B 510.)

With the object of ascertaining, by experimental inoculation, whether goats could be infected by the *M. melitensis*, six goats were bought on June 12, 1905, from two different herds, and placed in the lazaretto. On June 14 Dr. Zammit, as a preliminary step to our experimental work, took blood from each of these goats, and proceeded to test the action of the serum on the *M. melitensis*. He found, to his great surprise, that the serum of five of the goats, when considerably diluted, caused agglutination of this microbe. On June 15 similar results being again obtained, Dr. Zammit brought specimens of the bloods to the Public Health Laboratory, and asked me to confirm his observations. I obtained the following results:—

- Goat No. 1.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the *M. melitensis*, visible to the naked eye. When diluted 1 to 100, however, the serum gave no reaction.
- Goat No. 2.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the *M. melitensis*. A dilution of 1 to 100 produced a complete reaction after 15 minutes.
- Goat No. 3.—Blood serum diluted 1 to 10, 1 to 40, and 1 to 100, caused immediate agglutination of the *M. melitensis*, but, in the case of the dilution 1 to 100, the clumps were not visible to the naked eye until after 15 minutes.
- Goat No. 4.—The blood serum produced no reaction with the *M. melitensis*.
- Goat No. 5.—The blood serum diluted 1 to 10 caused immediate agglutination, but dilutions of 1 to 40 and 1 to 100 did not produce a complete reaction until after 15 minutes.
- Goat No. 6.—Blood serum diluted 1 to 300 caused complete agglutination of the *M. melitensis*, visible at once with the naked eye.

The reactions thus obtained, and especially that of Goat No. 6, suggested that possibly five of the goats were suffering from Mediterranean Fever, acquired under natural conditions. The goats were stated to be healthy, but were sold cheaply, as they had given very little milk for some time. They were bought from pens in the neighbourhood of Birchircara and St. Julians, and taken straight to the lazaretto, where they were placed in clean stalls, which had never been used for any experimental work with the *M. melitensis*.

Dr. Zammit and I then arranged to make a complete study of these animals; Dr. Zammit undertook the investigation of the blood, and I made myself responsible for the bacteriological examination of the milk and urine.

Bacteriological Examination of Milk and Urine obtained from Naturally Infected Goats.

Goat No. 6.—I commenced work with this goat, as its blood serum, when diluted 1 to 300, caused immediate agglutination of the *M. melitensis*. The animal did not appear well, and had a very poor coat. The udders were flaccid, but the milk exuded appeared normal in character. The temperature was taken morning and evening, and compared with that of a healthy goat. The evening temperature never rose above 103°, and, as this temperature is often recorded in the case of perfectly normal goats, a febrile temperature could not be said to be present. On June 18 milk was withdrawn, and 1 c.c. centrifugalised; the deposit was then carefully spread over 10 litmus-nutrose-agar plates. After four days' incubation at 37° C., colonies of the *M. melitensis* appeared in every plate. The colonies were at once tested with a dilute (1 to 100) specific serum obtained from an inoculated rabbit. The micrococci were found to agglutinate at once, the clumps being visible to the naked eye. Some of the colonies were then planted out on agar slopes, and the resulting growths, when subjected to the usual confirmatory tests, showed that the *M. melitensis* was undoubtedly being excreted in the milk of this goat.

On June 22 the milk was again examined and the *M. melitensis* recovered once more.

On June 23 examination of the urine was commenced. The vagina was washed out with an antiseptic solution and a catheter, previously sterilised in boiling water, passed into the bladder. The urine so obtained was plated on litmus-nutrose-agar, but after four days' incubation at 37° C., in spite of the precautions taken, the plates were found densely crowded with saprophytic organisms, and the *M. melitensis* could not be detected.

On June 24 and 26 the urine was again plated, the same precautions being used, but the plates were densely crowded with foreign organisms and the *M. melitensis* could not be seen.

On June 27, 28, 29, and 30, and on July 1, 3, 4, 5, 7, 8, 9, and 10, the urine was also examined, but up to the present the *M. melitensis* has not been recovered.

The milk was plated again in June and July, and the *M. melitensis* was found on each occasion.

Result.—The *M. melitensis* appears to be steadily excreted in the apparently normal milk of this goat, but up to the present it has not been found in the urine.

Goat No. 1.—This animal appeared healthy, but the udders were flaccid, and the milk exuded had a thin serous appearance. The temperature was taken regularly, but no indications of fever were observed.

On June 22, 1 c.c. of the milk was centrifugalised and the deposit plated. After four days' incubation at 37° C., the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24 and 26 the milk was again examined and similar results were obtained.

On June 26, 29, and 30 the urine was examined, but no signs of the *M. melitensis* could be discovered.

On July 1, 10 c.c. of the urine were centrifugalised and the deposit plated; four days later every plate was found studded with colonies of the specific microbe. The colonies were fished, planted on agar slopes, and the resulting growths tested in the usual manner.

Result.—The *M. melitensis* is excreted in very large numbers in the serous-looking milk of this goat. It is also excreted in the urine.

Goat No. 2.—This goat appeared quite well, and the milk exuded from the udders had a normal appearance. There were no indications of fever.

On June 22, 1 c.c. was centrifugalised and the deposit plated. After four days' incubation about 30 colonies appeared in every plate. On June 24 and 26 the milk was again examined, and colonies of the *M. melitensis* were recovered on both occasions.

The urine was examined on June 23, 26, 27, 28, 29, and 30, and on July 1, 3, and 6, but the *M. melitensis* could not be detected.

Result.—The *M. melitensis* appears to be excreted in small quantity in the normal-looking milk of this goat. It has not yet been detected in the urine.

Goat No. 3.—This goat looked healthy and had no fever, but its milk was thin and serous. On June 22 the milk was examined, one loopful of the serous milk being spread over each plate. After four days' incubation all the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24 and 26 the milk was again examined and similar results were again obtained.

The urine was examined on June 23, 26, 28, and 30, and on July 1, 3, 6, 8, 9, 10, 11, but no signs of the *M. melitensis* could be discovered.

Result.—The *M. melitensis* appears to be present in enormous quantities in the thin serous-looking milk of this goat, but it has not yet been found in the urine.

Goat No. 5.—This goat was in poor condition, the udders were flaccid, and the milk exuded had a thick jelly-like appearance.

On June 22 the milk was examined, one loopful of the jelly-like material being spread over each plate. After four days incubation all the plates were covered with minute colonies of the *M. melitensis*. On June 24 and 26 the milk was again examined, and densely crowded plates were obtained as before.

On June 25 and 30 the urine was examined, but no colonies of the *M. melitensis* were detected.

On July 1 the urine was again plated, and four days later every plate was found to contain numerous transparent colonies strongly resembling those of the *M. melitensis*. Some of the colonies were fished and planted out on agar slopes. The resulting growths were then subjected to the usual confirmatory tests, and the *M. melitensis* proved to be undoubtedly present.

The five goats just examined being considered by their owners to be "out of milk," would not be likely to be employed for milking purposes, though in the case of Goats Nos. 2 and 6, the milk might easily have been used without any fear of suspicion arising as to its being abnormal. Consequently it appeared very desirable to examine the herds which were actually supplying milk to Valetta, Sliema, and the various hospitals.

I therefore asked Captain Kennedy, R.A.M.C., to visit the various herds, and, with the owners' consent, take blood from the ears, and test the action of the sera on the *M. melitensis*. The results he obtained are given in Part VIII; it will be seen that, out of 161 goats examined, 84 gave a reaction, corresponding to a percentage of 52 probably infected with Mediterranean Fever. I then obtained samples of milk from some of the apparently infected animals, and proceeded to plate them on litmus-nutrose-agar. The following results have been obtained up to the present time :—

Examination of the Goats supplying Milk to Forrest Hospital.

I visited this herd, which assembles outside the hospital gate every morning, and selected Goats Nos. 38, 48, 37, and 43 from Captain Kennedy's list.

Goat No. 38.—The milk from this animal was centrifugalised, and the deposit plated on July 4, 5, 6, 7, 8, and 10, but, up to the present, the *M. melitensis* has not been isolated.

Goat No. 48.—The milk was examined on the same dates as Goat No. 38, but, so far, the *M. melitensis* has not been isolated.

Goat No. 37.—The milk of this animal was taken on July 4, and 2 c.c. centrifugalised; the deposit was then plated. After four days' incubation every plate was found densely crowded with small colonies of the *M. melitensis*; the colonies were so numerous that it was impossible to make an accurate count. The colonies were fished and planted on agar, the growths resulting responded to all the tests characteristic of the *M. melitensis*.

On July 5 and 6 the milk was again plated, and similar results were obtained.

As this goat was in full milk, there cannot be any doubt that the *M. melitensis* was being excreted in large numbers. A pint of the milk was then collected, and Dr. Zammit very kindly made a chemical examination of the sample. The result given below shows that the milk was of good quality.

Analysis of Milk from Goat No. 37.

Density at 15° C.	1030
Fat	4·3 per cent.
Total solids	13·18 „
Solids, non-fat	8·8 „
Ash	0·51 „

Goat No. 43.—The milk of this goat was examined on July 4, 5, 6, 7, 8, 9, and 10, but, up to the present, the *M. melitensis* has not been isolated.

A reference to Captain Kennedy's list shows that, while Goat No. 37 reacted in a dilution of 1 to 60, Goats Nos. 38, 48, and 43 only reacted in a dilution of 1 to 20, and were probably in an early stage of the disease.

Examination of a Small Herd supplying Milk to Valetta Station Hospital.

Goats Nos. 27, 30, and 32 were selected from this herd. The goats were kept at Casal Curmi, and brought every morning to the Station Hospital.

Goat No. 30.—On June 29 and 30 milk was centrifugalised and plated in the usual manner, but the *M. melitensis* was not detected.

On July 1 plates were again made, and a few typical colonies appeared.

On July 3 10 c.c. of the milk were centrifugalised, and the deposit plated; four days later every plate was found densely crowded with colonies of the *M. melitensis*.

On July 6 similar results were obtained.

A sample of the milk was then analysed by Dr. Zammit, and found to have an average chemical composition.

Goats Nos. 27 and 32.—The milk from these goats was examined on June 29 and 30, and on July 1, 3, 7, 8, and 10, but, up to the present, the *M. melitensis* has not been isolated.

Examination of a Small Herd Supplying Milk to Valetta.

This herd assembled in St. John's ditch, and 17 out of 25 animals showed a blood reaction with the *M. melitensis*, and six of them reacted when the serum was diluted 1 to 100. Goats Nos. 50 and 52 were selected from Captain Kennedy's list.

Goat No. 50.—On July 6, 1 c.c. of the milk was centrifugalised and the deposit spread over the usual plates. Four days later all the plates were found densely crowded with small colonies of *M. melitensis*.

The confirmatory tests were applied in the usual manner. This animal was considered one of the best milkers in the herd, and its owner valued it at £5, whereas the ordinary price for a goat in milk varies from £3 to £4.

Goat No. 52.—This animal appeared in good health and its udders were full of milk. It was purchased and placed in the lazaretto.

On July 5 milk was withdrawn and 1 c.c. centrifugalised; the deposit was then spread over nutrose-agar plates in the usual manner. After four days' incubation at 37° C., all the plates were found so crowded with colonies of *M. melitensis* that a reliable count could not be made.

On July 6 and 8 the milk was again examined and similar results were obtained.

A sample of the milk was submitted to Dr. Zammit for chemical analysis; he obtained the following results:—

Specific gravity at 15° C.....	1031
Total solids, 14·0 per cent. ; fat, 3·6 per cent. ; ash, 0·73 per cent.	

Examination of a Herd Supplying Milk to Sliema.

Two goats were bought from this herd and placed in the lazaretto. The pens were in the neighbourhood of Misida.

Goat No. 15.—On July 5 the blood was examined and the serum, diluted 1 to 50, was found to cause complete agglutination of the *M. melitensis* visible to the naked eye. The goat appeared to be in good health, and the udders were full of milk. Some milk was withdrawn and 2 c.c. centrifugalised; the deposit was then plated in the usual manner. On July 9 the plates were found covered with small colonies of the *M. melitensis*.

On July 6 the milk was again examined, and the deposit from

90 Goats as a Means of Propagation of Mediterranean Fever.

1 c.c. produced as before an immense number of colonies of *M. melitensis*.

The urine was withdrawn by a catheter and plated on July 5, 6, 7, 8, 9, and 10, but up to the present the *M. melitensis* has not been isolated.

A chemical analysis of the milk was made by Dr. Micallef, with the following results:—

Total solids, 13·5 per cent. ; fat, 4·1 per cent. ; ash, 0·75 per cent.

Goat No. 16.—This goat was taken from the same herd as No. 15. On July 4 the blood was examined, and the serum diluted 1 to 60, was found to cause immediate clumping of the *M. melitensis*. The milk and urine have been examined daily since July 4, but up to the present the *M. melitensis* has not been isolated from either source.

Conclusions.—The results obtained show that some of the goats in every herd examined are suffering from Mediterranean Fever. The *M. melitensis* is exuded in the milk in enormous numbers when the disease has been present sufficiently long to cause a change in the physical characters of the fluid. It is also excreted in considerable numbers even when the animals are in "full milk," and no changes have occurred in either the physical or chemical characters of the milk.

The *M. melitensis* is also excreted in the urine of goats suffering from Mediterranean Fever, but up to the present it has only been found when the disease has existed for some time and physical changes have occurred in the milk.

VIII. EXAMINATION OF GOATS' BLOOD FOR REACTION TO MEDITERRANEAN FEVER.

By J. CRAWFORD KENNEDY, R.A.M.C., Member of the Mediterranean Fever Commission, Malta.

No. of herd.	Owner and number in each herd.	Address.	Milk supplied to—	Total number examined.	Number that gave no reaction.	Number that reacted to Med. Fever.	Per-centage of reactions.	Table showing amount of reaction in each infected goat by dilutions up to 1:100.						
								Dilution.	10.	20.	40.	60.	80.	100.
1	C—, Nos. 1 to 4 and 74 to 83	Casal Tar- shiel near C a s a l Paulo	Cottonera Hospital, Zabbar Gate and near lying part of town	14	7	7	50	No. of goat { 2 74 80 78 Total ...	2	74 78	1 75	83
2	A—M— and F—G—, Nos. 5 to 17	Zabbar ...	Cottonera Hospital, Zabbar Gate and near lying town	13	12	1	7·6	No. of goat { ... Total	5
3	J—, Nos. 18 to 20	Hamrun ...	Valetta ...	3	3	No. of goat { ... Total	1
4	J—F—, Nos. 21 to 23	C a s a l Curmi	Valetta Hospital and town	13	9	4	30·7	No. of goat { ... 32 ... 30 Total	32 30	...	32	...	27
								No. of goat { ... Total	2	...	1	...	1

No. of herd	Owner and number in each herd.	Address.	Milk supplied to—	Total number examined.	Number that gave no reaction.	Number that reacted to Med. Fever.	Per-centage of reactions.	Table showing amount of reaction in each infected goat by dilutions up to 100.								
								Dilution.	10.	20.	40.	60.	80.	100.		
5	C—, Nos. 34 to 48	St. George's	Forrest Hospital ...	15	10	5	33·3	{	No. of goat	...	38	...	37
									Total	4	...	1	
									No. of goat	51	54	...	64	...	50	
										59	66	55	
6	M—M—, Nos. 40 to 73	St. John's Ditch	Valetta ...	25	8	17	69	{	No. of goat	63	70	60	
									65	68		
									73	71		
									Total ...	6	4	...	1	...	6	
7	G—M—, Nos. 84 to 129	Hamrun ...	Valetta ...	46	20	26	56	{	No. of goat	97	88	85	103	...	99	
									109	93	90	111	...	102		
									117	96	...	119	...	105		
									120	106	...	126	...	121		
7	G—M—, Nos. 84 to 129	Hamrun ...	Valetta ...	46	20	26	56	{	No. of goat	123	110	122	
									...	112	129		
									...	113		
									...	128		
7	G—M—, Nos. 84 to 129	Hamrun ...	Valetta ...	46	20	26	56	{	Total ...	5	9	2	4	...	6	
									Total ...	5	9	2	4	...	6	

8	F— M—, Nos. 130 to 161	Pietà	...	Valette	...	32	8	24	75	No. of goat	137	134	131	147	...	133
											140	135	137	133
											144	141	136
											148	149	138
											145
											151
											153
											154
											155
											156
											159
											160
											161
Total ...											4	4	2	1	...	13
Total ...											52.17					
Total ...											84					
Total ...											77					
Total ...											161					

Examination of a Herd of Goats kept Privately and not allowed outside their own Field, as a Comparison with the Herds that Walk into Town Daily.

Total examined	10
No reaction	5
React to Mediterranean Fever	5

Of the five which reacted :—

1 reacted in dilution	$\frac{1}{10}$
3 " " "	$\frac{3}{10}$
1 " " "	$\frac{1}{100}$

∴ The percentage of infected goats in this herd is 50 per cent., comparing very closely with 52 per cent. of the public herds.

IX. RESULTS OF EXAMINATIONS FOR THE ISOLATION OF *MICROCOCCUS MELITENSIS* FROM THE BLOOD, URINE, AND SPUTUM OF CASES INFECTED WITH MEDITERRANEAN FEVER IN HASLAR HOSPITAL.

By P. W. BASSETT-SMITH, Fleet-Surgeon, Haslar.

Blood.—The blood was obtained from the median basilic vein of the arm, which had been carefully sterilised; from 1 to 3 c.c. were taken with an all-glass anti-toxin syringe; the blood was at once injected into flasks containing 50 c.c. of peptone broth; from this sub-cultures were made on to agar daily for 14 days at least, the resulting growth being tested by—

- (1) Agglutination with specific serum.
- (2) Alkaline reaction with litmus milk.
- (3) Negative staining by Gram.

In all 27 bloods were examined from 24 patients, with 16 positive and 11 negative results, as shown in the following table :—

No.	Day of disease.	Condition.	Temperature.	Amount of blood taken.	Result.
			° F.	c.c.	
1	50	Acute relapse	102·4	3	Positive
2	142	Slight "	99·4	2·5	"
3	84	Acute "	102	3	"
4	117	" "	103·4	3	"
5	92	" "	102	3	"
6	23	Prim. wave	104	3	"
7	34	Sec. "	103	3	"
8	167	Relapse.....	102	1	Negative
		short relapse with neuritis			
9	143	Acute relapse	101	2	Positive
10	44	" "	101·2	2·5	"
11	105	" "	102·6	3	"
12	153	" "	105	2	"
13	44	" "	102·8	0·5	"
14	41	" "	102	2	"
15	80	" "	102·6	2	"
16	111	" "	101	2	"
17	61	" "	103	2	"
18	122	Convalescent	N.	1	Negative
19	185	Cachexia.....	N.	2	"
20	110	Slight relapse	100	3	"
		slight wave, not repeated			

No.	Day of disease.	Condition.	Temperature. ° F.	Amount of blood taken. c.c.	Result.
21	165	Convalescent	N.	3	Negative went out next day
22	159	„	N.	3	Negative no return of the fever
23	130	„	N.	3	Negative
24	78	„	N.	3	„ no return of the fever
25	134	„	N.	3	Negative no fever, great anæmia
26	120	„	99	8	Negative flask contaminated on the 14th day
27	1 year	Cachexia	N.	3	Negative

Two flasks after being inoculated were found to be contaminated and are not included in this list, the others were either sterile or contained a pure culture of *M. melitensis*.

Excepting for cases 8, 20, and 26, the *M. melitensis* was recovered from all cases examined where fever was present. When not found I think the prognosis is favourable for continued convalescence.

Urines.—The technique as recommended by Major Horrocks was followed—the penis being washed with carbolic acid solution, the urine collected in a sterile test-tube after a part had been passed to clear the urethra, and then 0·2 c.c. plated on litmus-glucose-nutrose-agar, incubated at 37° C.

In all 46 urines have been thus examined, of 18 patients, with the following results. In the great majority of the plates, in 24 hours the surface was covered by a spreading foul-smelling acid organism, or was thickly studded with opaque rapidly growing colonies of a rather large coccus, but in two instances typical colonies of *M. melitensis* were present as a pure culture, both being from the same patient.

No. of case.	No. of examinations.	Condition.	Result.
1	1	Acute relapse	Negative
2	7	" "	"
3	1	" " T. 103	"
4	1	" "	"
5	2	" "	"
6	5	" "	"
7	3	" "	"
8	1	Convalescent	"
9	7	"	"
10	2	Acute relapse	"
11	2	" "	"
12	2	" "	"
13	1	" "	"
14	1	" "	"
15	4	" "	"
16	2	" "	"
17	11	" "	<div style="display: inline-block; vertical-align: middle;"> 1 with 16 col. on plate 1 with 14 col. on plate, pure culture of <i>M. melitensis</i> 9 rapidly overgrown </div>
18	1	" "	Negative

Unless the urine is free from other organisms, there seemed to be little chance of isolating the *M. melitensis*, the growth of this organism being so much slower than most of the others present.

Case 16 was very acute, in a typhoid condition, and the urine was drawn off with a catheter, but was full of other organisms.

Sputum.—As several of the cases recently received into Haslar were suffering from bronchial catarrh, with expectoration of mucoid sputum, I thought it possible that in these one might be able to isolate the organism from the sputum.

Technique.—The sputum in the early morning was received into a sterile test-tube, a fragment of the thickest portion was then fished out on a platinum loop, thoroughly washed in a tube of sterilised water, and again removed to a second test-tube of sterile water, thoroughly mixed, and 0·2 c.c. plated out on nutrose-glucose-litmus-agar, incubated at 37°.

So far no colonies resembling the *M. melitensis* have been met with, and as all the cases have ceased to expectorate, the experiment has ceased.

No. of case.	No. of experiment.	Condition.	Character of sputum.	Result.
1	1	Acute relapse	Muco. purt.	Negative
	2	" "	"	"
2	3	Convalescent	Mucoid	"
3	4	Acute relapse	"	"
4	5	" "	"	"
	6	" "	"	"
5	7	" "	"	"
	8	" "	"	"
	9	" "	"	"
6	10	" " T. 104.....	"	"

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REPORTS
OF THE
COMMISSION
APPOINTED BY
THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,
FOR THE INVESTIGATION OF
MEDITERRANEAN FEVER,
UNDER THE SUPERVISION OF AN
ADVISORY COMMITTEE
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I. FURTHER NOTES ON THE ISOLATION OF THE *MICROCOCCUS MELITENSIS* FROM PERIPHERAL BLOOD; AND EXPERIMENTS ON THE DURATION OF LIFE OF THIS MICROBE IN EARTH AND IN WATER.

By Staff-Surgeon R. T. GILMOUR, R.N.

(Received August 28, 1905.)

The method of procedure employed was similar to that described in my previous paper.* The cases selected for this series of experiments had well-marked symptoms of Mediterranean Fever, and in no case was the agglutination reaction less than 1 in 50. Control experiments were made to ascertain whether the microbe was present in relatively large quantities of blood. From 1 to 3 c.c. of blood and 30 to 50 c.c. of broth were used for this purpose.

Cases XX and XXXIV are interesting. In the former the *Micrococcus melitensis* was isolated during a relapse on or about the 300th day from the date of the patient first going sick. The latter is a case of Mediterranean Fever occurring during convalescence from enteric.

CASE XX, D. J., Lieutenant, R.N.—This officer was admitted into hospital in December, 1903, for Mediterranean Fever. The symptoms were well marked, with an agglutination reaction of 1 in 200. He was discharged to duty in January, 1904. In April, 1904, he forwarded me his serum, and as it only reacted 1 in 10, I gave him a favourable prognosis. At the end of September, 1904, he got very wet whilst out shooting in the Greek Islands, and went sick about 14 days afterwards with fever. On admission into hospital on October 15, 1904, he had well-marked Mediterranean Fever, with an agglutination reaction 1 in 100; later, 1 in 400. On November 4, 1904, about the 300th day since date of first going sick, a pure growth of *Micrococcus melitensis* was recovered from his blood.

CASE XXXIV, J. C.—This man was received into hospital with well-marked symptoms of enteric fever: dry, brown tongue, diarrhoea, intestinal hæmorrhage, and an agglutination reaction of 1 in 400 to the *B. typhosus* and negative to *Micrococcus melitensis*. On November 6, 1904, the 7th day of disease, 3 c.c. of blood were extracted from the right median basilic vein and disposed of as follows:—1 c.c. was passed

* Reports of the Commission, Part I, p. 78.

into 19 c.c. of broth (A), 1 c.c. into 9 c.c. (B), and 2 c.c. into 50 c.c. (C). From (A), broth tubes were inoculated with various quantities of emulsion, containing from 0.0025 to 0.05 c.c. of blood. From (B), plate cultures were inoculated with 1 c.c. of emulsion (0.1 c.c. of blood). These tubes and plates remained sterile. Flask (C) showed turbidity after 24 hours, and by the 2nd day contained a profuse growth of the typhoid bacillus. The fever ran an ordinary course, but when the temperature had remained normal for 15 days it again rose, and patient's serum was now found to react both to *Micrococcus melitensis* and *B. typhosus* 1 in 50. On January 20, 1905, the 82nd day of the enteric attack, blood cultivation gave a pure growth of the *Micrococcus melitensis*.

In an experience of two years and a half with Malta Fever I have never found enteric and Mediterranean Fever running concurrently, but I have come across several cases of the latter immediately following the former.

Deductions to be drawn from these experiments :—

(1) That *Micrococcus melitensis* is found in the peripheral blood of about 82 per cent. of cases of Mediterranean Fever. In the 45 cases examined, no control was made in 8, and in 1 the cultures were contaminated. Of the remaining 36 the microbe was isolated in 29, and also in 1 with no control.

(2) That the number of *Micrococcus melitensis* per cubic centimetre of blood is small, rarely reaching 100. Out of the 30 cases quoted above, in only 6 did they number 100 or more. Largest number found, 400 per cubic centimetre.

(3) That the *Micrococcus melitensis* can be recovered as early as the 2nd day and as late as the 300th day of the disease.

(4) That patients convalescent from enteric may contract Mediterranean Fever, so that the former disease does not appear to confer immunity against the latter.

The experiments (p. 5) were undertaken at the suggestion of the Committee of the Mediterranean Fever Commission. The results obtained were similar to those of Horrocks' and Bassett-Smith, with the exception of the life of *Micrococcus melitensis* in sea-water. In sterile sea-water it appeared to me to exist only for a very short time—about 13 days; in non-sterile sea-water the plates were so crowded out with other bacteria that I failed to isolate it after the 1st day.

The following deductions can be drawn from these experiments :—

1. That the *Micrococcus melitensis* can exist for considerable periods outside the body, but that it does not multiply.
2. That it can exist in dry, sterile garden soil for at least 60 days.
3. In sterile tap-water for at least 42 days.
4. In sterile sea-water for at least 13 days.
5. In non-sterile tap-water for at least 7 days.

Experiment.	Day of disease.	Approximate number of <i>Micrococcus melitensis</i> per c.c. of blood.
XIV	6	0·0
XV	189	0·0
XVI	16	40·0
XVII	18	Present in control
XVIII	32	0·0
XIX	40	0·0
XX	300	Present in control
XXI	7	0·0
XXII	2	Present in control
XXIII	9	0·0
XXIV	29	Present in control
XXV	8	20·0
XXVI	3	Present in control
XXVII	12	" "
XXVIII	49	" "
XXIX	34	" "
XXX	30	" "
XXXI	10	400·0
XXXII	40	Present in control
XXXIII	45	" "
XXXIV	?	" "
XXXV	7	0·0
XXXVI	12	50·0
XXXVII	5	0·0
XXXVIII	180	0·0
XXXIX	40	333·3
XL	4	0·0
XLI	11	100·0
XLII	17	0·0
XLIII	15	100·0
XLIV	6	Present in control
XLV	12	" "

A. Duration of Life of *Micrococcus melitensis* in Dry, Sterile Garden Soil.

Experiment 1.—February 15, 1905. The soil was inoculated with 1 c.c. of an eight-day-old broth sub-culture. The flask was then placed in a dark cupboard, and shaken frequently, the soil being allowed to dry naturally.

Micrococcus melitensis was recovered on the 60th day.

B. *Duration of Life of Micrococcus melitensis in Non-sterile Tap-water.*

Experiment 1.—February 2, 1905. One hundred cubic centimetres of tap-water were inoculated with the whole of an eight-day-old agar slope culture.

Micrococcus melitensis was isolated on the 7th day.

C. *Duration of Life of Micrococcus melitensis in Sterile Tap-water.*

Experiment 1.—February 3, 1905. Fifty cubic centimetres of tap-water were sterilised by heating to 120° C. in an autoclave for 30 minutes, and were then inoculated with the whole of an eight-day-old agar slope. The flask was kept in a dark cupboard.

The *Micrococcus melitensis* was recovered up to the 6th day.

Experiment 2.—This experiment was repeated with a four-day-old sub-culture.

Micrococcus melitensis was recovered up to the 42nd day.

Experiment 3.—February 8, 1905. Ten cubic centimetres of tap-water, sterilised in the autoclave at 115° C. for twenty minutes, were inoculated with the whole of a five-day-old agar sub-culture.

Micrococcus melitensis was recovered on the 19th day, but not later.

Experiment 4.—This experiment was again repeated. The agar sub-culture used was 12 days old.

Micrococcus melitensis was recovered on the 23rd day.

D. *Duration of Life of Micrococcus melitensis in Non-sterilised Tank-water.*

Experiment 1.—February 15, 1905. The water was obtained from a foul drinking tank, contaminated with large quantities of animal and vegetable matter.

The *Micrococcus melitensis* could not be recovered two days after inoculation.

E. *Duration of Life of the Micrococcus melitensis in Sterilised Tank-water.*

Experiment 1.—February 15, 1905. The same water was used as in the last experiment, but in this case it was sterilised for 30 minutes at 100° C.

Micrococcus melitensis was recovered on the 12th day after inoculation.

F. Duration of Life of Micrococcus melitensis in Tank-mud.

Experiment 1.—February 15, 1905. Mud, unsterilised. A glass flask was filled to a depth of one inch with fairly liquid mud, which was inoculated with 5 c.c. of a 12-day-old broth culture.

The *Micrococcus melitensis* could not be recovered from this unsterilised mud two days after inoculation.

G. Duration of Life of Micrococcus melitensis in Sterilised Tank-mud.

Experiment 1.—Mud sterilised.

The *Micrococcus melitensis* was recovered from this sterile mud on the 21st day after inoculation.

H. Duration of Life of Micrococcus melitensis in Unsterilised and Sterile Sea-water.

Experiment 1.—February 2, 1905. One hundred cubic centimetres of unsterilised sea-water were inoculated with the whole of an eight-day-old agar slope.

The microbe was recovered from non-sterile sea-water one day after inoculation, but not afterwards.

Experiments 2, 3 and 4.—February 24, 1905. One hundred cubic centimetres of sea-water were heated to 120° C. in an autoclave for 15 minutes, and were then inoculated with the whole of a nine-day-old agar slope.

Micrococcus melitensis was recovered from No. 2 on the 13th; from No. 3 on the 13th, and from No. 4 on the 12th day after inoculation.

II. THE AMBULATORY TYPE OF CASE IN MEDITERRANEAN OR MALTA FEVER.

By Staff-Surgeon E. A. SHAW, R.N.

(Received November 25, 1905.)

The existence of this type of case amongst a people with whom a specific fever has been for scores of years endemic had long been surmised. The importance of such cases as sources of infection has been amply demonstrated by Koch in his anti-typhoid campaign in the Rhine provinces in 1902, but with regard to Malta Fever the existence of such cases has hitherto been merely a matter of conjecture and not of absolute knowledge.

Accordingly in June of 1905, I set myself to the task of investigating the existence or otherwise of this type of case of Malta Fever amongst the Maltese. For this purpose it was deemed necessary to have available a large number of Maltese actually in full work, each readily identifiable, and under control, so that any one individual could be readily got at for the necessary observations. The method contemplated was to examine the blood of a considerable number for agglutination reaction, and further to make a bacteriological examination of the blood and urine for *Micrococcus melitensis* of such individuals as might present a well-marked agglutination reaction. For obvious reasons women were not contemplated as subjects for the investigation. It was felt to be highly probable that there would be considerable difficulty in getting even a sufficient number of men to submit voluntarily to the necessary procedure.

Having regard to various possibilities, I considered that the Naval Dockyard in Malta, which employs several thousands of Maltese, and gets over the difficulty of a frequently-recurring identity of name by allotting to each man a number, offered the best field for this inquiry. I accordingly obtained from Admiral Bromley, the Admiral-Superintendent of the Dockyard, an authority to proceed as I proposed. It was arranged that the various heads of departments should send batches of men, told off without discrimination, to the Dockyard Surgery on days to be arranged between us for the purpose of having samples of their blood taken for the ascertaining of agglutination reaction.

With the most cordially rendered assistance of Fleet-Surgeon Hardie, R.N., and Surgeon Westcott, R.N., I was able to obtain specimens of blood in capillary tubes from 525 dockyard employes. Each tube had a flag label attached to it bearing the man's name and dockyard number, and corresponding lists of names and numbers were prepared

as the men came up to have their fingers pricked. I next proceeded to examine these 525 samples of blood for agglutination reaction to *Micrococcus melitensis*, using a dilution of 1/30 of each for that purpose. This was a somewhat laborious undertaking, and my thanks are due to Major Horrocks and Captain Kennedy, who very kindly examined between them some 140 of the whole number of samples.

As the result of this preliminary examination it was found that 79 out of the 525, or 15 per cent., gave a distinct agglutination reaction with *Micrococcus melitensis*. Of these 79, a marked reaction was presented by 22, which were accordingly selected for a detailed bacteriological examination of both the blood and urine of the men on the following conditions.

Blood.—Bend of elbow sterilised, 5 c.c. of blood taken from median basilic vein and placed in 80 c.c. of nutrient broth in a flask, this well shaken and placed in incubator at 37° C., daily agitated; sub-cultures made on to agar slopes on 6th and on 11th days. If no result appeared on 14th day, the investigation was abandoned as unfruitful; if a growth appeared, it was put through the usual tests for *Micrococcus melitensis*, and the result recorded. Blood was not taken more than once in each case, owing to the dislike of the subject to the operation.

Urine.—A supply of sterilised test-tubes was daily sent to the Dockyard surgery; the men selected were told to call there each morning at 7 A.M. on entering the dockyard to commence their work. The surgery attendants were instructed (1) to see that each man cleansed the meatus urinarius and glans with 1 in 40 carbolic solution, and (2) to collect the first 1 oz. of urine passed in a suitable vessel for rejection, and the second 1 oz. in one of the sterilised test-tubes, which was then inscribed with the man's number and the date. The samples thus obtained were to be sent to me in the laboratory, where I immediately proceeded to plate each out. From each daily sample of urine two Petri dishes containing nutrose litmus agar (no glucose), of a reaction +10, were inoculated, 1/3 of a c.c. (six drops, about) being distributed over the surface of each with a spreader. These plates were numbered, dated, and incubated at 37° C. for six days. They were then examined and the likely colonies were put through the usual tests for *Micrococcus melitensis*. Where, as in Cases IX and XI, the colonies of this organism were too numerous to be counted individually, the numbers given were arrived at by selecting an average area, counting the colonies contained in 1 sq. cm. of this, and determining the total number of square centimetres covered with colonies. The urines were thus daily examined for 28 successive days, exclusive of Sundays.

I will now describe these 22 cases as briefly as possible. The temperatures given are those of each man for the first few days of the observations, the first being the morning and the second the evening

temperature. All these men were in full work for the whole period of these observations, with the exception of Case I, who was at home once for three days on the sick list. For the temperatures and brief details of each case, my thanks are due to Fleet-Surgeon Hardie, R.N., of H.M. Dockyard at Malta, who tells me that he found it impossible to ascertain definitely what kind of fever it was which some of these men say they had previously had.

CASE I.—G. Araci, 4112, age 25, labourer. Had a week's fever about 12 months ago.

Temperatures.— $99^{\circ}/99^{\circ}\cdot 2$; $98^{\circ}\cdot 6/99^{\circ}\cdot 4$; $99^{\circ}/99^{\circ}\cdot 8$; $99^{\circ}\cdot 2/99^{\circ}\cdot 6$; away 3 days; $99^{\circ}/99^{\circ}\cdot 2$.

Blood yielded no *Micrococcus melitensis*.

Urine yielded 3 colonies of *Micrococcus melitensis* on July 4.

2	"	"	"	"	12.
23	"	"	"	"	19.
3	"	"	"	"	21.

CASE II.—G. Busutil, 1918, age 17, boiler-maker's apprentice. Had fever 13 years ago.

Temperatures.— $98^{\circ}/99^{\circ}$; $98^{\circ}\cdot 4/99^{\circ}$; $98^{\circ}\cdot 6/98^{\circ}\cdot 2$; $98^{\circ}/98^{\circ}\cdot 8$; $98^{\circ}\cdot 4/98^{\circ}\cdot 6$; $98^{\circ}/98^{\circ}\cdot 4$; $98^{\circ}\cdot 2/98^{\circ}\cdot 4$.

Blood yielded no *Micrococcus melitensis*.

Urine " " "

CASE III.—G. Ciantar, 3528, aged 38, joiner. Was sick for 2 days 2 months ago.

Temperatures.— $99^{\circ}\cdot 8/100^{\circ}$; $99^{\circ}\cdot 6/99^{\circ}\cdot 8$; $98^{\circ}\cdot 4/98^{\circ}\cdot 2$; $98^{\circ}\cdot 0/98^{\circ}\cdot 8$; $98^{\circ}\cdot 0/98^{\circ}\cdot 4$; $98^{\circ}\cdot 4/98^{\circ}\cdot 6$; $98^{\circ}\cdot 2/98^{\circ}\cdot 4$.

Blood yielded *Micrococcus melitensis*.

Urine yielded 1 colony of this organism on July 6.

3 colonies of	"	7.
5	"	8.
3	"	13.
10	"	15.
3	"	17.
5	"	18.
10	"	19.
6	"	21.
1 colony	"	24.

CASE IV.—F. Darmanin, 4221, age 30, hammerman. Had fever 12 years ago.

Temperatures.— $99^{\circ}\cdot 8/99^{\circ}\cdot 4$; $99^{\circ}\cdot 6/99^{\circ}\cdot 2$; $98^{\circ}\cdot 6/98^{\circ}\cdot 0$; $98^{\circ}\cdot 2/98^{\circ}\cdot 6$; $98^{\circ}\cdot 4/98^{\circ}\cdot 4$; $98^{\circ}\cdot 0/98^{\circ}\cdot 2$; $98^{\circ}\cdot 2/98^{\circ}\cdot 4$.

Blood yielded no *Micrococcus melitensis*.

Urine yielded 3 colonies of *Micrococcus melitensis* on July 14.

1 colony of	"	21.
9 colonies of	"	24.

CASE V.—C. Cassar, 1203, age 28, assistant fitter. Has had headaches and giddiness for the last 3 months.

Temperatures. — $99^{\circ}0/99^{\circ}0$; $98^{\circ}0/98^{\circ}2$; $98^{\circ}/98^{\circ}$; $98^{\circ}0/98^{\circ}2$; $98^{\circ}0/98^{\circ}2$; $98^{\circ}2/98^{\circ}4$; $98^{\circ}0/98^{\circ}4$.

Blood yielded no *Micrococcus melitensis*.

Urine

CASE VI.—T. Sceberas, 3475, age 35, joiner. Had fever in April and May last.

Temperatures. — $99^{\circ}/99^{\circ}$; $98^{\circ}6/99^{\circ}0$; $98^{\circ}4/98^{\circ}2$; $98^{\circ}0/98^{\circ}6$; $98^{\circ}/99^{\circ}$; $98^{\circ}/98^{\circ}$; $98^{\circ}6/98^{\circ}2$.

Blood yielded no *Micrococcus melitensis*.

Urine

CASE VII.—F. Grima, 1686, age 50, founder. No history of fever. Complains of "general debility."

Temperatures. — $100^{\circ}/101^{\circ}2$; $99^{\circ}4/99^{\circ}4$; $99^{\circ}/98^{\circ}$; $98^{\circ}/98^{\circ}$; $98^{\circ}4/98^{\circ}0$; $98^{\circ}/98^{\circ}$; $99^{\circ}2/100^{\circ}$.

Blood contained *Micrococcus melitensis*.

Urine yielded 9 colonies of *Micrococcus melitensis* on July 11.

1 colony	"	"	12.
1	"	"	15.
2 colonies	"	"	20.
13	"	"	21.
3	"	"	25.

CASE VIII.—D. Burlo, 1094, age 21, assistant fitter. Had "fever," lasting 15 days 2 years ago.

Temperatures.— $98^{\circ}0/98^{\circ}8$; $99^{\circ}0/98^{\circ}6$; $98^{\circ}6/98^{\circ}2$; $98^{\circ}4/98^{\circ}6$; $99^{\circ}2/98^{\circ}0$; $98^{\circ}2/98^{\circ}2$; $98^{\circ}/98^{\circ}$.

Blood yielded no *Micrococcus melitensis*.

Urine

CASE IX.—B. Worley, 1857, age 29, boilermaker. Had fever 10 months ago.

Temperatures.— $98^{\circ}0/98^{\circ}4$; $98^{\circ}0/98^{\circ}4$; $98^{\circ}2/98^{\circ}6$; $98^{\circ}0/98^{\circ}8$; $98^{\circ}4/98^{\circ}4$; $98^{\circ}0/98^{\circ}4$; $98^{\circ}0/98^{\circ}2$.

Blood yielded no *Micrococcus melitensis*.

Urine contained *Micrococcus melitensis* every day from July 3 to end of August, usually in very large quantity. It was then arranged to examine it twice weekly; it has been present on every occasion up to date of writing (November 21). The periodical examination is still being continued. Enumerations of the colonies were made on following dates in the manner described:—

July 5, 26,631 colonies of *Micrococcus melitensis* per c.c. urine.

7,	57	"	"
10,	16,023	"	"
14,	24	"	"
22,	22,869	"	"

July 24,	32,319 colonies of <i>Micrococcus melitensis</i> per c.c. urine.		
August 9,	9,450	"	"
14,	69	"	"
26,	381	"	"
September 29,	690	"	"
October 10,	9,005	"	"

This man's urine has been used for various feeding and other experiments with monkeys and goats by both Major Horrocks and myself, and has infected both these species of animal.

CASE X.—V. Borg, 3567, age 34, tailor. Had "fever" for 3 weeks about 10 or 11 months ago.

Temperatures.—99°·0/98°·4; 98°·8/99°·2; 99°·2/98°·8; 98°·6/99°·2; 99°·0/98°·4; 98°·0/98°·2; 98°·4/98°·4.

Blood did not yield *Micrococcus melitensis*.

Urine

CASE XI.—F. Mallia, 3414, age 31, joiner. Had fever for 4 weeks, commencing May 1, 1905.

Temperatures.—98°·8/99°·0; 98°·6/98°·6; 98°·4/98°·4; 98°·6/98°·0; 98°·2/98°·4; 98°·6/98°·4; 98°·6/98°·2.

Blood contained *Micrococcus melitensis*.

Urine contained this organism every day from July 3 to end of August. During September and October it was plated twice weekly, and only failed to yield *Micrococcus melitensis* on one occasion (September 12), when the plates were bad. The number of colonies per cubic centimetre of urine have not been so numerous as in Case IX, but have varied within wider limits as follows:—

July 3,	39 colonies of <i>Micrococcus melitensis</i> per c.c. urine.		
11,	291	"	"
13,	981	"	"
22,	108	"	"
24,	1,953	"	"
August 16,	6,426	"	"
25,	270	"	"
September 29,	13,380	"	"
October 18,	1,017	"	"

This man's urine also has been used for various successful animal infection experiments (q.v.), and up to date of writing (November 21) has continued to yield *Micrococcus melitensis* from each of the bi-weekly samples.

CASE XII.—G. Sacoett, 3326, age 15, rivet boy. Had 2 days' illness with headaches 4 months ago.

Temperatures.—98°·8/99°·0; 98°·4/99°·2; 98°·4/98°·6; 98°·2/98°·2; 98°·2/98°·4; 98°/98°; 98°·2/98°·4.

Blood did not yield *Micrococcus melitensis*.

Urine " "

CASE XIII.—G. Bianco, 2565, age 46, shipwright. Four years ago had fever for 2 months followed by arthritis.

Temperatures.—98°·6/98°·4; 98°·6/98°·4; 99°·0/98°·6; 98°·0/98°·8; 98°·0/99°·0; 98°·2/98°·0; 98°·0/98°·2.

Blood did not yield *Micrococcus melitensis*.

Urine " "

CASE XIV.—G. Cutajar, 3046, age 27, shipwright. States he has never had fever.

Temperatures.—99°·8/99°·2; 98°·0/99°·2; 98°·8/99°·0; 98°·4/98°·4; 98°·2/99°·0; 98°·4/98°·2; 98°·2/99°·0.

Blood did not yield *Micrococcus melitensis*.

Urine " "

CASE XV.—G. de Giovanni, 3022, age 27, shipwright. Three years ago had fever for about 3 weeks.

Temperatures.—98°·4/98°·4; 98°·0/98°·4; 98°/99°; 98°·4/98°·6; 98°/99°; 98°·2/98°·8; 98°·0/98°·4.

Blood did not yield *Micrococcus melitensis*.

Urine " "

CASE XVI.—G. Parlar, 3797, age 16, rivet boy. Had slight fever 8 months ago.

Temperatures.—98°·2/99°·0; 98°·6/98°·6; 98°·0/98°·8; 98°·0/99°·2; 98°·0/98°·6; 98°·2/98°·2; 98°/98°.

Blood did not yield *Micrococcus melitensis*.

Urine yielded 2 colonies of *Micrococcus melitensis* on August 3.

2	"	"	8.
5	"	"	14.
7	"	"	26.

CASE XVII.—Carmelo de Celis, 3796, age 15, rivet boy. Had 10 days' fever a fortnight ago.

Temperatures.—98°·4/100°·4; 98°·2/99°·8; 98°·6/99°·2; 99°·0/99°·8; 98°·8/100°; 98°·4/99°·2; 98°·0/99°·4.

Blood contained *Micrococcus melitensis*.

Urine did not yield this organism.

CASE XVIII.—E. Casinguena, 2911, age 33, shipwright. Had 10 weeks' fever 14 months ago.

Temperatures.—98°/98°; 98°·2/98°·4; 98°/98°; 98°·2/98°·4; 98°/98°; 98°·2/98°·6; 98°·0/98°·4.

Blood did not contain *Micrococcus melitensis*.

Urine yielded 1 colony of *Micrococcus melitensis* on August 4.

5 colonies	"	"	10.
2	"	"	16.
3	"	"	19.

CASE XIX.—A. Ghirsci, 3111, age 40, shipwright. Never had fever.

Temperatures. — $98^{\circ}/98^{\circ}$; $98^{\circ}0/98^{\circ}4$; $98^{\circ}0/98^{\circ}8$; $98^{\circ}6/98^{\circ}4$; $98^{\circ}0/98^{\circ}8$; $98^{\circ}/98^{\circ}$; $98^{\circ}4/98^{\circ}6$.

Blood did not yield *Micrococcus melitensis*.

Urine " "

CASE XX.—R. Mamo, 906, age 32, assistant fitter. Had fever 2 years ago for 3 months.

Temperatures. — $98^{\circ}0/98^{\circ}8$; $98^{\circ}0/98^{\circ}4$; $98^{\circ}/98^{\circ}$; $98^{\circ}2/98^{\circ}6$; $98^{\circ}4/98^{\circ}2$; $98^{\circ}0/98^{\circ}6$; $98^{\circ}2/98^{\circ}4$.

Blood did not yield *Micrococcus melitensis*.

Urine yielded 3 colonies of *Micrococcus melitensis* on August 8.

2	"	"	12.
8	"	"	18.

CASE XXI.—E. Agius, 2550, age 58, shipwright. Never had fever.

Temperatures. — $99^{\circ}/99^{\circ}$; $99^{\circ}0/99^{\circ}4$; $99^{\circ}8/99^{\circ}2$; $98^{\circ}8/99^{\circ}6$; $98^{\circ}4/99^{\circ}2$; $98^{\circ}6/98^{\circ}4$; $98^{\circ}4/98^{\circ}6$.

Blood did not yield *Micrococcus melitensis*.

Urine " "

CASE XXII.—A. Gatt, 3625, age 49, painter. Had slight fever for about 4 days 2 years ago.

Temperatures. — $99^{\circ}/99^{\circ}$; $98^{\circ}4/99^{\circ}6$; $98^{\circ}4/99^{\circ}2$; $98^{\circ}8/99^{\circ}0$; $98^{\circ}4/99^{\circ}0$; $98^{\circ}8/99^{\circ}2$; $98^{\circ}4/98^{\circ}4$.

Blood did not yield *Micrococcus melitensis*.

Urine " "

Results.—Thus all 22 of these cases gave a marked agglutination reaction with *Micrococcus melitensis*. From three of them (Cases III, VII, and XI) the parasite was recovered from both blood and urine. From one it was obtained from the blood only (Case XVII), and from six (Cases I, IV, IX, XVI, XVIII, and XX) from the urine only. All these men were up and about and in full work during the period of observation.

Though all these men presented such a marked agglutination reaction as to make it a certainty, they had all had Malta Fever some time or other; Cases VII, XIV, XIX, and XXI deny ever having had it at all, although from Case VII *Micrococcus melitensis* was recovered from both blood and urine. As regards temperatures, it will be seen there was a slight rise above the normal in three of the four (III, VII and XVII), from whose blood the *Micrococcus melitensis* was recovered; that of the fourth (Case XI) being practically continuously normal. While of those six from whom the urine alone yielded the parasite, the temperatures of two (Cases I and IV) were slightly raised, those of the remaining four (Cases IX, XVI, XVIII and XX) being practically normal. The infectivity of the urines of these cases is shown by the fact that a

few cubic centimetres of the urine of No. 9, injected subcutaneously into a monkey, gave rise to a typical attack of Malta Fever, with recovery of *Micrococcus melitensis* from the blood and organs, and also by the successful feeding experiments on both monkeys and goats (q.v.) which were carried on separately by Major Horrocks and myself with the urines of Cases IX and XI, which excreted *Micrococcus melitensis* on a remarkable scale both in regard to amount and duration.

- Conclusions* 1.—That the existence of ambulatory cases is now proved.
2.—That their urine contains *Micrococcus melitensis*, in large quantity and for prolonged periods, is proved.
3.—That their urine is a source of infection, at least to monkeys and goats, is proved.
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III. MEDITERRANEAN FEVER IN GOATS, COWS, AND OTHER ANIMALS.

By Staff-Surgeon E. A. SHAW, R.N.

(Received November 25, 1905.)

I. GOATS.

A.—*Experimental.*

To determine experimentally to what degree goats, which are so numerous in Malta, are susceptible to Malta Fever, I determined, in July, 1904, to inject cultures of *Micrococcus melitensis* subcutaneously into these animals. Thinking that possibly there might be a difference of susceptibility between the mature and immature animal, I began with experiments on a goat and a kid. On July 30, 1904, a female goat, two years old, and a female kid, three months old, were purchased, their blood was examined for agglutination reaction to *Micrococcus melitensis* (which was found to be nil), and they were kept under observation for a week. They were then dealt with as follows:—*

Experiment 1.—Goat ♀. July 30, 1904, no agglutination reaction; temperature normal, practically between 101° and 102° F. till August 8, 1904. Injected subcutaneously at noon of that day into left flank an emulsion of the six-day growth of *Micrococcus melitensis* on six agar slopes, second generation from spleen of human fatal case. This caused a rise of temperature of 3°·8 F., from 102° to 105°·9 F. on the 9th, and 107°·2 on the 10th of August, then gradually falling back to normal by August 14.

Agglutination.—Agglutination reaction was first present on August 13, 1 in 30; rising to 1 in 200 on August 14, and 1 in 1800 on August 20, on which date the goat received a second injection of the growth from four similar agar slopes of *Micrococcus melitensis*, which caused a similar rise of temperature for three days. On August 29 it received a third injection of emulsified growth from four more slopes. On August 30 the agglutination reaction was 1 in 2200, and on September 5, 1 in 3200. All these were visible under $\frac{2}{3}$ -in. objective in 15 minutes.

* [It should be stated that the first of these experiments was briefly described by the author in the manuscript of the Report by him published in the first Part of the Reports of this Commission (March, 1905), but the paragraph was deleted at his request, as the observations were, in his opinion, still incomplete, though he recognised and stated that the "goats, which are extremely numerous in Malta, might possibly be instrumental in transmitting the infection of Malta Fever."—Sec. R.S.]

Urine.—This was plated daily, after having been drawn off into a sterile vessel by a sterile catheter, which I passed myself from August 25 to September 6, $\frac{1}{2}$ c.c. of urine being distributed over the surface of glucose nutrose litmus agar contained in Petri dishes, which were incubated for six days at 37° C. On September 6, 1904, I went on three weeks' leave. On my return experiments were resumed by me on September 30. A fourth subcutaneous injection of *Micrococcus melitensis* growth from six agar slopes as before was made on October 10, with the idea of giving the goats' kidneys plenty of the parasite to excrete. The experiment was continued till October 31 and was then given up, no *Micrococcus melitensis* having been at any time recovered from the urine of this animal.

Milk.—This was first plated for recovery of possible *Micrococcus melitensis* on August 28, 1904; but on September 1 the plates were found to be completely overgrown with a *Staphylococcus*, and on centrifugalising the milk from each udder and making film preparations from the deposit, I recognised pus in the milk from each udder. On August 28 also, feeding experiments with the milk of this goat were commenced on a healthy monkey (No. 61), a stomach tube being passed and 1 oz. given on this date, 2 ozs. on August 29, 4 ozs. on the 30th, and 4 ozs. on the 31st; but such severe diarrhoea was developed that no more milk was given after the last date. This monkey, unfortunately, succumbed on September 4. The usual inoculations were made from its organs and heart's blood into broth, and on to agar slopes, but no *Micrococcus melitensis* was recovered. The milk of this goat was examined from time to time, but the pus persisted till April 25, 1905, by which time the milk was "drying up." It may be noticed in passing that suppurative mastitis is by no means infrequent amongst goats in Malta and causes, from time to time, outbreaks of illness amongst children (see *Health Reports of Malta*). In June, 1905, the secretion of milk had practically ceased and pus was no longer present in the altered secretion, now thick, ropy, brownish and gelatinous; which, on being plated on June 24, yielded colonies of *Micrococcus melitensis* in abundance.*

Blood.—The agglutination reaction continued to increase, being 1 in 3200 on September 30, 1904, and 1 in 4500 on October 18. It then began to diminish, being 1 in 3000 on November 1, 1904, 1 in 3000 on January 3, 1905, 1 in 3000 on February 27, 1905, and after this stationary period going down to 1 in 2000 on April 25, 1905, and 1 in 1500 on June 12, 1905.

Micrococcus melitensis was recovered from the blood (5 c.c.) of the

* [Major Horrocks had previously recovered *Micrococcus melitensis* from apparently normal goats, and had shown the plates to Staff-Surgeon Shaw.—S.c. R.S.]

jugular vein on November 7, 1904, and again from the blood of the same vein on June 27, 1905.

Experiment 2.—Goat, juv. The kid of three months, purchased at the same time as the goat, received injections of *Micrococcus melitensis* on the same dates as the goat, but only in half the quantities.

Its urine was plated on the same days above detailed for the goat but it never yielded any *Micrococcus melitensis*. Its agglutination reaction was similarly examined with the following results :—

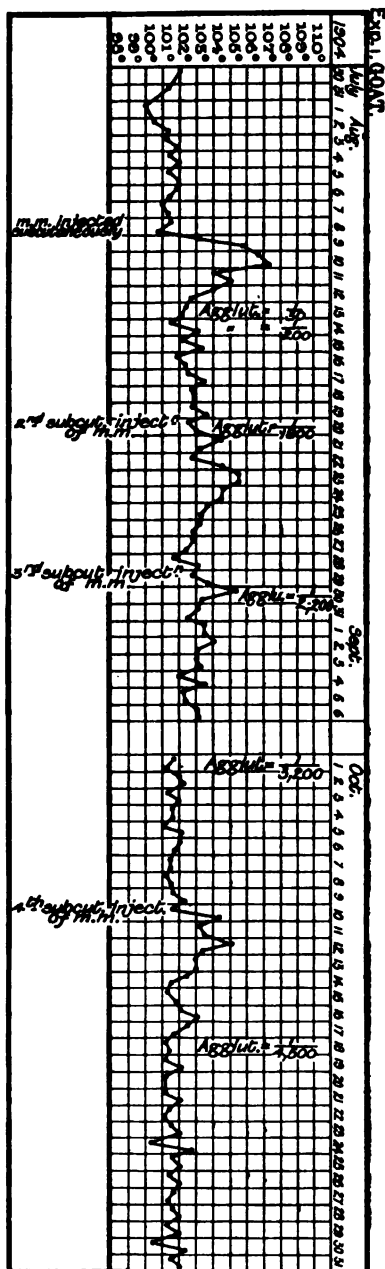
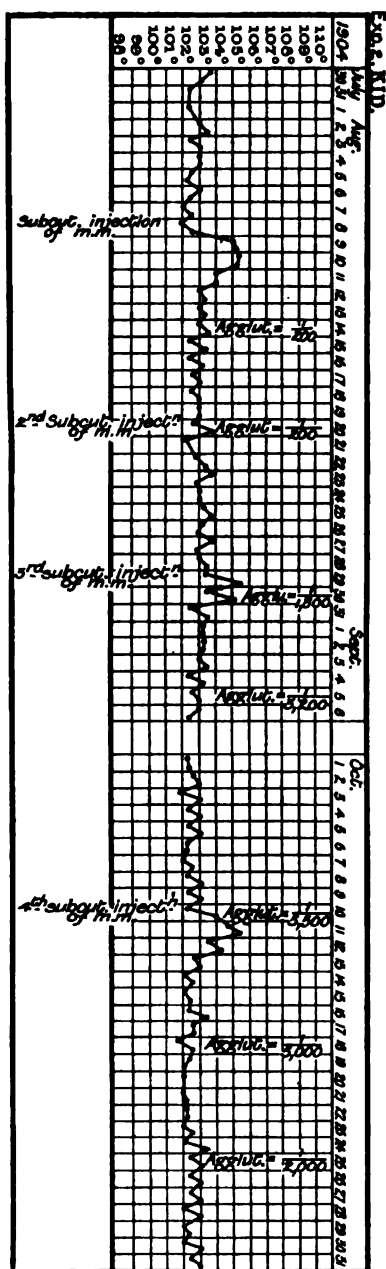
1904—		
July	30 Nil
August	14 1/200
„	20 1/200
„	30 1/1800
September	5 1/3200
„	30 1/3200
October	11 1/5500
„	18 1/5000
„	25 1/2000
November	1 1/3000
1905—		
January	3 1/2000
February	27 1/1500
April	25 1/1500

These figures follow closely those obtained from the goat, but rise somewhat higher in degree. *Micrococcus melitensis* was recovered from the blood of the kid in November, 1904, and in June, 1905.

From an inspection of the accompanying charts it will be seen that the rise of temperature following each injection is only temporary, lasting but three or four days, thus decidedly differing from the prolonged wave of fever produced in monkeys by a similar procedure, and suggesting a sort of racial tolerance of this infection on the part of the Maltese goat. It was thus proved by the development of a high agglutination reaction in August, 1904, and by the recovery of living *Micrococcus melitensis* from the blood in November, 1904, that the goat is at least experimentally susceptible to Mediterranean Fever.

B. Natural.

Dr. Zammit in June, 1905, found that the blood of four goats (out of six purchased for further experimentation) reacted to *Micrococcus melitensis* at the time of purchase. This observation having been confirmed by Major Horrocks and myself, and the micro-organism having also been recovered from the milk of one of these animals, the question of the agency of the goat in diffusing the *Micrococcus melitensis* was brought from the domain of speculative experiment



into the range of everyday life.* And after discussing the matter with Deputy Inspector-General Bentham, of the Naval Hospital at Bighi, we decided that I should at once begin an examination of all the goats supplying milk to this hospital.

The method of procedure adopted was as follows:—The goats were taken in batches of 12 to 16 at a time. The milk contractor's son took down in Maltese the name and description of each goat, which was numbered. A little blood was taken from the goat's ear in a capillary tube for examination for agglutination, and at the same time 40 to 50 c.c. of milk were drawn off into a sterile test-tube; to both of these the same number was attached, the intention being to subsequently eliminate all the goats which might be found infected. After examination of the blood for agglutination reaction, the milk of each goat which reacted was centrifugalised, and the centrifuged portion was plated on nutrose-litmus-agar, $\frac{1}{4}$ c.c. being distributed over the surface of each Petri dish. These were incubated at 37° C., and were then examined for *Micrococcus melitensis* colonies in the usual way. Four to six plates were used for each milk thus treated.

The examination was begun on June 29. Surgeon Whiteside, R.N., was so good as to collect the necessary material from the goats at times when I was unable to attend, and he also helped with the agglutination reactions. These were done in two stages, the first to eliminate the non-agglutinating bloods (or milks), the second to determine highest dilution giving agglutination of the remainder. All went well for a few days, until the goat-herds, who had all along looked unhappy over the pricking of their goats' ears for blood, broke out into open rebellion, and henceforth we had to be content with only milk. This necessitated either plating every milk or ascertaining the existence of the agglutination reaction with it. I have already mentioned the excretion of agglutinins in the urine (Part III of these reports, p. 47). It seemed not unlikely that they would be found to be present in the milk of infected animals. I accordingly put up specimens of such milk, centrifuged and uncentrifuged, diluted and undiluted, with freshly prepared emulsion of *Micrococcus melitensis* in drops on slides in a moist chamber, with controls of normal milk, and left them for an hour. I then examined them under the microscope and found distinct agglutinations with the infected milks; most palpably in the uncentrifuged specimens, the appearance in the centrifuged series being somewhat masked by *débris* of various sorts.

* Major Horrocks states that Dr. Zammit and he found that five of the normal goats reacted, and that he recovered *Micrococcus melitensis* from the milk of all of them but one. He remarks, further, that "the fact that the milk of infected goats causes agglutination of the *Micrococcus melitensis* was first shown by Zammit, and in the combined paper by Kennedy and myself the reaction is called Zammit's test."
—[Sec. R.S.]

When the milk was allowed to stand in the sterile test-tubes for a couple of hours, a considerable layer of cream came to the top and a deposit of *débris* gathered at the bottom. By passing a pipette down to the middle of the column, aspirating milk from there, withdrawing the pipette and then breaking off the capillary end of the pipette well above the adherent cream, I could obtain a specimen of milk almost free from *débris* and with relatively few oil globules, in which the presence or absence of agglutination was fairly easily determinable. This method was, therefore, perforce adopted for the ascertaining of agglutination reaction in the batches of goats examined on July 13 and 14, a 24-hour limit for contact of diluted milk and emulsion being adopted for determination of highest dilution giving agglutination:

The details of these examinations of the goats supplying milk to Bigli Hospital in June and July, 1905, are subjoined.

June 29. Twelve goats examined. Agglutination reaction found in blood of three: No. 4, in a dilution of 1 in 30; No. 8, 1 in 100; No. 10, 1 in 60. Milks of all three centrifugalised, plated, and incubated. *Micrococcus melitensis* recovered and verified from No. 8 only (49 colonies).

July 3. Second batch of 12 goats examined. Agglutination reaction found in blood of five: No. 4, 1 in 100; No. 5, 1 in 60; No. 6, 1 in 60; No. 8, 1 in 30; No. 9, 1 in 30. Milks of all these five centrifugalised, plated, and incubated. *Micrococcus melitensis* recovered and verified from No. 5 (38 colonies), and No. 6 (728 colonies).

July 6. Third batch of 16 goats examined. Agglutination reaction found in blood of three: No. 1, 1 in 100; No. 3, 1 in 60; No. 14, 1 in 160. Milks of these three centrifugalised, plated, and incubated. *Micrococcus melitensis* recovered and verified from No. 3 (six colonies).

July 7. Fourth batch of 12 goats examined. Agglutination reaction found in blood of three: No. 5, 1 in 30; No. 7, 1 in 30; No. 10, 1 in 30. Milks of these three centrifugalised, plated, and incubated. No *Micrococcus melitensis* recovered from any.

July 10. Fifth batch of 12 goats examined. Agglutination-reaction found in blood of six: No. 1, 1 in 60; No. 5, 1 in 200; No. 7, 1 in 200; No. 8, 1 in 60; No. 9, 1 in 60. Milks of these six centrifugalised, plated, and incubated. *Micrococcus melitensis* recovered and verified from No. 1 (10 colonies) and from No. 7 (seven colonies).

July 13. Sixth batch of 15 goats examined. Agglutination reaction found in milk of four: No. 2, 1 in 100 after 24 hours' contact in moist chamber; No. 11, 1 in 60; No. 14, 1 in 30; and No. 15, 1 in 30, all under the same conditions. Milks of these four centrifugalised, plated, and incubated. *Micrococcus melitensis* recovered from No. 2 (15 colonies) and No. 11 (five colonies).

July 14. Seventh batch of 12 goats examined. Agglutination

reaction found in milk of six : No. 1, 1 in 150 after 24 hours' contact in moist chamber; No. 2, 1 in 160; No. 4, 1 in 30; No. 7, 1 in 30; No. 9, 1 in 30; No. 12, 1 in 30. *Micrococcus melitensis* was recovered and verified only from No. 4 (two colonies).

For convenience of reference, these results may be arranged in tabular form, thus :—

Date.	No. of goats examined.	No. presenting agglutination reaction.	Distinguishing No. of the goats of each batch whose milk yielded <i>Micrococcus melitensis</i> .	Agglutination limit of latter.	No. of <i>Micrococcus melitensis</i> colonies recovered.
29 June...	12	3	No. 8	1 in 100	49
3 July...	12	5	No. 5	1 " 60	38
6 " ...	16	3	No. 6	1 " 60	728
7 " ...	12	3	No. 3	1 " 60	6
10 " ...	12	6	None	—	—
			No. 1	1 in 60	10
			No. 7	1 " 200	7
13 " ...	15	4	No. 2	1 " 100	15
14 " ...	12	6	No. 11	1 " 60	5
			No. 4	1 " 30	2
Totals...	91	30	9	—	—

Thus 91 goats were examined, of these, 30 presented the agglutinating reaction on *Micrococcus melitensis*, and the milk of these 30 was examined culturally for the parasite, this organism being recovered from the milk of nine of them. The implicated animals were eliminated from the herds supplying the Naval Hospital, and the most stringent measures were taken to ensure that all milk entering the hospital gates was forthwith boiled. It will be interesting to see whether any alteration takes place in the future incidence of cases of fever developing in this hospital.

It will be noticed that in these naturally-infected goats the agglutination limit is low, the highest found being 1 in 200, whereas in the experimentally infected animal it was found as high as 1 in 4500. No indication has been observed of any relation between agglutination value and the number of colonies of *Micrococcus melitensis* yielded by the milk.

The number of organisms other than *Micrococcus melitensis* found in the milk from these 30 goats varied enormously, though the milk was collected under precisely similar conditions from all. In some cases $\frac{1}{4}$ c.c. of milk would contain but two or three organisms, in others they would be present by the thousand. Time did not admit of a detailed examination being made of these.

The infectivity of the milks obtained from the goats which were the subjects of the experiments here detailed was investigated as follows:—

A monkey received from Genoa on July 12 was kept under observation for a week, its temperature was found to be normal during this period, and its blood did not react to *Micrococcus melitensis*. On July 20, the colonies of this micro-organism obtained from No. 2 goat's milk, plated July 13, were emulsified in a little normal saline solution. The monkey being held on its back, three drops of this emulsion were dropped down each nostril with a capillary pipette. The animal developed a typical attack of Mediterranean Fever, its blood gave agglutination reaction to *Micrococcus melitensis* first on August 3; 14 days after infection in a dilution of 1 in 30, running up to 1 in 320 on August 6, and 1 in 960 on August 10; *Micrococcus melitensis* was recovered from its blood during life on August 22, and from its lymphatic glands after death on October 18.

II. Cows.

As it seemed by no means impossible that cows also might be found to be infected with Mediterranean Fever, I determined to investigate this question. Not many milch cows are to be seen in the Island of Malta, there being no pasturage for them. Their owners keep them shut up, some of their stables being most scrupulously clean, while others are much the reverse. The cows seldom get outside. There is a considerable demand for their milk, especially on the part of the resident English population, many of whom dislike the taste of goat's milk, while others object to receive milk from an animal which has just previously been lying down in the street with its udders and teats in close contact with the excreta, liquid and solid, of the various animals, higher and lower.

To Mr. A. M. Macfarlane, M.R.C.V.S., Veterinary Surgeon to the Malta Government, who helped me to procure the necessary materials for bacteriological examination, my warmest thanks are due for the kindness with which he took me round to the various farms, used his influence with the owners of the cattle, and personally assisted in collecting the necessary material for examination.

The method of investigation determined on was as follows: At each of the various farms visited, blood was taken from the cows, each animal being assigned a number, which was cut deep in Roman numerals into the hair of its back. The samples of blood were correspondingly numbered, and were subsequently examined for agglutination reaction to *Micrococcus melitensis*. The numbers of the cows, at each farm, giving this reaction, were sent with a daily supply of sterilised test tubes to Mr. Macfarlane, who undertook the collection

of a daily sample of milk from each of the cows specified. These milks were received at the laboratory about an hour after they were drawn. They were at once centrifugalised, and the deposit was plated on nutrose-litmus-agar in Petri dishes, three plates to each sample. These were incubated five days at 37° C., and were examined in the usual way for colonies of the micro-organism. The milks were thus treated daily from August 1 to August 24, 1905, inclusive, with the following results:—

G. F. of Tarrien.—Nine cows, of which three presented agglutination reaction to *Micrococcus melitensis* as follows: No. 3, 1 in 30; this being a heifer, no milk was attainable, and no other form of examination was permitted. No. 4 agglutinated *Micrococcus melitensis* in a dilution of 1 in 30, and No. 7 in a dilution of 1 in 60. The milks of the two latter were daily plated for 24 consecutive days. Cow No. 7 never yielded any colonies of parasite, but these were found in the milk of Cow No. 4, as follows:—

Plates of 7th August 5 colonies of *Micrococcus melitensis*.

"	8th	"	7	"	"
"	12th	"	7	"	"
"	16th	"	3	"	"
"	19th	"	40	"	"
"	20th	"	3	"	"
"	21st	"	39	"	"
"	24th	"	19	"	"

none being found on the other days.

F. G. of Hamrun.—Nine cows, of which five presented agglutination reaction as follows:—

No. 1 agglutinated 1 in 30.

" 4 " 1 in 800.

" 8 " 1 in 200.

" 9 " 1 in 30.

Of these Cow No. 9 was ailing and not yielding any milk. The milks of the others were plated daily. Nos. 1 and 4 never yielded any colonies of *Micrococcus melitensis*, which, however, were found in the milk of Cow No. 8, as follows:—

Plates of 11th August, 63 colonies of *Micrococcus melitensis*.

"	12th	"	9	"	"
"	13th	"	31	"	"
"	16th	"	23	"	"
"	18th	"	7	"	"
"	19th	"	13	"	"
"	20th	"	231	"	"

none being found on the other days.

S. G. of Hamrun.—Six cows, none of which presented any agglutination reaction.

C. G. of Hamrun.—Three cows, of which only 1 presented an agglutination reaction in a dilution of 1 in 30. The milk of this animal was daily examined, but never presented any *Micrococcus melitensis*.

C. C. of St. Julian's.—Four cows, of which No. 2 presented a high agglutination reaction, this being present in a dilution of 1 in 1000. Unfortunately this animal happened to be a heifer, so again no further material for bacteriological examination was procurable.

S. M. of Imsieral.—Two cows, neither of which presented any agglutination reaction to *Micrococcus melitensis*.

Result.—Thirty-three cows examined. Ten of these presented an agglutination reaction to *Micrococcus melitensis*, varying from 1 in 30 to 1 in 1000. From the milk of two of these cows *Micrococcus melitensis* was isolated.

III. OTHER ANIMALS.

During the months of July and August, 1905, I examined specimens of blood kindly procured for me by Mr. Macfarlane, M.R.C.V.S., from 31 bullocks which were ailing in a vague indefinite sort of way, and which he thought might possibly be infected with Malta Fever. Of these, five presented a very faint agglutination reaction. None of these animals had been in the island over three months.

I also examined several times the blood of two dogs similarly suspected. Neither of these presented any reaction to *Micrococcus melitensis*.

REMARKS.

The manner in which animals become infected with the virus of Mediterranean Fever is a matter of considerable interest and importance. Up to the present all the evidence available points to their food as being the main vehicle of infection. The feeding experiments carried on by Major Horrocks and by myself show conclusively that monkeys and goats may be thus infected. Besides the very obvious way of infection of the young through their mothers' milk, the successful result of various feeding experiments with food soiled, directly and indirectly, with the urine of two ambulatory cases of Mediterranean Fever which I discovered working in the Dockyard, and in whose urine living *Micrococcus melitensis* was being excreted, indicates another way in which these animals may be infected while feeding. Goats may be seen any day in the streets of the chief city of the Island of Malta feeding on filth and rubbish of every possible variety, some of it visibly saturated with urine, animal and human. Among

the lower class Maltese, as above stated, workmen have been found who void living *Micrococcus melitensis* in their urine, as do a certain number of the infected goats. Thus the path of this manner of infection becomes clear. Having satisfied their hunger in this manner, the goats lie down in the streets to digest their meal with their teats and udders often in contact with the ordure of the gutters and roads, till they are kicked up by the goat-herd to be milked into the vessel brought to the doors of the adjacent houses by their occupants. It is hence not to be wondered at that these animals frequently suffer also from suppurative mastitis, and give milk containing pus. In the Health Reports of the Malta Government may be seen reports of outbreaks of illness amongst children directly traced to this cause by their medical officers.

With regard to cows, the evidence is not quite so clear. Kept shut up in "shippens," and seldom allowed outside, they have their food brought to them, but as this food is composed of vegetable and other refuse collected from every possible source and situation, it is easy to understand that they can hardly escape from receiving infected food from time to time.

Summary.

1. The susceptibility of goats to experimental infection by *Micrococcus melitensis* was ascertained by me in the summer of 1904, and is here further demonstrated.

2. The persistence of living *Micrococci* in the blood of a goat for seven months has been proved. The bearing of this observation on the preparation of a therapeutic serum is obvious.

3. Of 91 goats in full milk, 30 were found to have become infected with Mediterranean Fever at some time or other, as shown by their agglutinating power on *Micrococcus melitensis*. Living examples of the micro-organism were recovered from the milk of nine of these, and its infectivity was demonstrated on a monkey.

4. Of 33 cows examined, 10 were found to have become infected with Mediterranean Fever, and living *Micrococcus melitensis* was recovered from the milk of two of these.

5. Of 31 bullocks examined, five were found to show a faint agglutination reaction, which may indicate that they had become infected with Malta Fever.

6. Of two ailing dogs, thought to be suffering from this fever, neither was found to be infected.

IV. ON THE DURATION OF LIFE OF THE *MICROCOCCUS MELITENSIS* IN UNSTERILISED SOIL.

By Major W. H. HORROCKS, R.A.M.C.

(Received December 16, 1905.)

In Part I of the "Reports of the Mediterranean Fever Commission," studies were described as to the duration of life of the *Micrococcus melitensis* in sterilised soils, and it was shown that not only did the organism survive in these soils, but also that it was possible to infect monkeys by causing them to inhale infected dust. These results having been obtained, it became necessary to ascertain whether the microbe could live in unsterilised dust such as is found in the streets of Malta, and also in earth fouled with excrementitious material.

Experiment I.

To ascertain the duration of life of the *Micrococcus melitensis* in dust collected from the Strada Mercanti, Malta.

On May 19 dust was collected from the street and placed in sterile test-tubes. A loopful of the dust was then added to varying quantities of water contained in sterile watch glasses. Surface plates were next made from these dilutions, and the dilution determined which produced colonies sufficiently discrete, after four days' incubation; to enable the *Micrococcus melitensis* to be detected should it be present.

On May 22 an emulsion of an agar growth of the *Micrococcus melitensis* in sterile water was added to the street dust so as to wet every particle, the tubes were then placed in the laboratory cupboard and allowed to dry naturally.

On May 27 the dilution of the soil determined above was plated on the surface of 12 glucose-litmus-nutrose-agar plates. Typical colonies, reacting to all the tests, were recovered.

On June 13 the *Micrococcus melitensis* was again isolated.

On June 19 a successful recovery was also made, but after this all attempts to isolate the microbe were unsuccessful.

Result.—The *Micrococcus melitensis* lived for 28 days in natural dust collected from the Strada Mercanti.

Experiment II.

To ascertain the duration of life of the *Micrococcus melitensis* in unsterile building dust.

This particular dust was selected as it is often polluted by the workmen during building operations, and, being very light, is easily blown about the streets.

On May 22 the dust was placed in sterile test-tubes, and then thoroughly moistened with an emulsion of an agar growth of the

Micrococcus melitensis in sterile water. As in Experiment I, trial plates were made to ascertain the quantity of soil which would produce discrete colonies on surface glucose-litmus-nutrose-agar plates.

On May 24 and 27, and on June 2, 5, 9, 13, and 19 the *Micrococcus melitensis* was isolated from the soil, but all the attempts to recover it at later dates proved unsuccessful.

Result.—The *Micrococcus melitensis* lived for 28 days in the natural building dust found in the streets of Valetta.

Experiment III.

To ascertain the duration in life in soil of a culture derived from *Micrococcus melitensis* which had assumed a saprophytic existence for some seven weeks.

It was thought that a *Micrococcus melitensis*, which had apparently become accommodated to conditions of life external to the human body, might live for a much longer period in soil than a culture directly isolated from the spleen of a Mediterranean Fever patient. Accordingly, on June 22, a culture of the microbe, which had been isolated from a sample of tap-water to which it had been added seven weeks previously, was made into an emulsion with water and then added to dust swept up from the Strada Mercanti. The same procedure was followed as in Experiment II. The *Micrococcus melitensis* was recovered on the twenty-fifth day after the commencement of the experiment, but not at a later date.

Result.—A culture of a *Micrococcus melitensis*, which had already assumed a saprophytic existence, did not appear to live longer in soil than a culture freshly isolated from the body of Mediterranean Fever patients.

Experiment IV.

To ascertain the duration of life of the *Micrococcus melitensis* in non-sterile manured garden soil.

Soil obtained from a garden which had been recently manured was placed in test-tubes, and then thoroughly wetted with an emulsion in water of an agar culture of *Micrococcus melitensis* isolated from a case of Mediterranean Fever. The same procedure was followed as in Experiments II and III, but, owing to the foul condition of the soil, it was found that only a few particles of soil could be used to make a workable dilution. The microbe was recovered five days after the inoculation was made.

Experiment V.

This was a repetition of Experiment IV. The *Micrococcus melitensis* survived for 20 days.

Having ascertained that the *Micrococcus melitensis* could live for three to four weeks in polluted soils, it appeared desirable to ascertain

whether the *Micrococcus melitensis* could be recovered from a soil infected with the urine of Mediterranean Fever patients. Accordingly, a urine containing from 10,000 to 30,000 micrococci per cubic centimetre was added to building dust and to dust swept up from the Strada Mercanti, and attempts were made to isolate the specific organism by the procedure already detailed. Unfortunately, all the experiments failed, and the reason is not far to seek. Polluted soils contain from 5,000,000 to 50,000,000 microbes per gramme, and taking the urine at its richest and the soil at its lowest computation, there would be only 3 of the specific micrococci to 500 of the soil microbes. A plate containing 500 colonies is far too crowded to allow of the *Micrococcus melitensis* being isolated, as, after four days' incubation at 37° C., such a plate is completely overgrown. Further dilutions were attempted, but the *Micrococcus melitensis* could not be recovered.

Experiments were then made to ascertain the duration of life of the *Micrococcus melitensis* when a sterilised soil is infected with the urine from Mediterranean Fever patients. The same procedure was followed as in the other experiments, and the *Micrococcus melitensis* was readily isolated after 24 hours, but not at a later date. It was found that the saprophytic microbes in the urine rapidly multiplied in the soils experimented with, and after 48 hours the plates became unworkable unless a dilution of the soil, which practically precluded all hope of recovering the *Micrococcus melitensis*, was employed.

Experiments made to Determine whether it is possible to Infect Animals with Dust Polluted by Urine of Mediterranean Fever Patients.

In the Report of the Commission, Part I, experiments were related which showed that monkeys could be infected by dust artificially inoculated with large quantities of the *Micrococcus melitensis*, and in view of the "dust theory" in relation to the question of the infection of human beings, it appeared important to repeat the experiments, using, however, a dust infected with urine containing the *Micrococcus melitensis*. Such experiments would be the nearest approach that could be made to the conditions actually occurring in Nature.

Experiment I.—Infected Dust blown into the Throat and added to Food.

Monkey No. 111 was kept under observation for some 14 days, and its temperature taken morning and evening. Its blood was also repeatedly examined for reaction with the *Micrococcus melitensis*. The animal appearing perfectly well, the experiment was commenced on July 21, when dust from the Strada Mercanti, infected with urine from Case No. XI (Shaw), and then dried for four days at 37° C., was blown into the pharynx. On the next day a little of the dust was mixed with a feed of boiled rice. The same procedure was

followed until August 9, when, no trace of infection having appeared, it was determined to dry the dust for only 24 hours at 37° C., as it was thought that the prolonged drying in the incubator might have destroyed the virulence of the microbe. On August 13 artificially dried dust was discarded, and dust dried naturally at the room temperature employed. On August 18 the monkey was seized with an acute attack of diarrhoea, and died on the 22nd. At the *post-mortem* examination nothing abnormal was noticed. Cultures were made from the spleen, liver, kidneys, heart's blood, mesenteric, femoral, and axillary glands. All the cultures, however, proved to be sterile.

Experiment II.—Infected Dust blown in the Nostril.

Monkey No. 112 was kept under observation and examined as in the previous experiment. On July 22 sterile building dust, infected with urine of Case XI, and dried artificially, was injected into the nostril. The same dust was injected daily until July 26, when a dust infected with the mixed urine of Cases IX and XI was employed. The injection of this dust once a day having also failed to produce any sign of infection, on August 9 a dust dried at the laboratory temperature was used. On August 23 the monkey appeared very ill, and refused his food; there was, however, no fever, and the blood showed no signs of a reaction with the *Micrococcus melitensis*. The dust injections were discontinued, and the monkey slowly improved, but he never regained his former sleek appearance.

On October 15 the blood diluted 1/10 gave a positive reaction.

On October 18 severe dropsy developed, and the monkey, being seriously ill, was killed with chloroform. Nothing abnormal was found at the *post-mortem* examination. Cultures were made from the spleen, liver, kidneys, bile, blood, and glands, but no signs of the *Micrococcus melitensis* were discovered.

Experiment III.—Infected Dry Dust added to Food.

Monkey No. 113 was kept under observation for a fortnight, and its blood was repeatedly tested as to agglutination with the *Micrococcus melitensis*. On July 24 dust infected with urine of Case XI, and dried naturally for four days, was added to the food.

On July 26 dust infected with the mixed urine of Cases IX and XI was added to the food. The feeding was continued daily until August 6, when the monkey was seized with violent vomiting and diarrhoea, and died on the following morning. During the experiment the blood was repeatedly examined, but never showed the slightest trace of a reaction with the *Micrococcus melitensis*. At the *post-mortem* examination fluid was found in the peritoneum and pericardium; the liver, spleen, and kidneys appeared congested. Cultures were made

from the spleen, liver, kidneys, bile, heart's blood, and glands. No signs of the *Micrococcus melitensis* were detected.

Experiment IV.—Infected Dry Dust added to Food.

Monkey No. 114 was used for this experiment, preliminary observation having shown that it was perfectly healthy. On July 24 dust infected with the urine of Case XI, and dried naturally for 10 days, was added to the food. The feeding was continued daily until August 9, when, owing to the high external temperature, it was found possible to dry the soil in 24 hours. Feeding with this soil was continued until September 17. The blood was examined weekly from the commencement of the feeding, but never showed the slightest trace of a reaction with the *Micrococcus melitensis*. The monkey is still under observation, and appears in perfect health.

Remarks.—Though the attempts to infect monkeys with dust polluted with urine from a case of Mediterranean Fever have failed, the histories of Goats Nos. 13 and 14 show that it is possible to infect these animals in this manner. The ingestion of polluted soils gave rise to violent vomiting and diarrhoea in the case of the three out of the four monkeys experimented upon, and this disturbance of the digestive tract may possibly account for the failure of the experiments. Monkey No. 114 may have been insusceptible. Monkeys vary considerably in their susceptibility to infection; No. 110, mentioned in later experiments, could not be infected, though it received the growth on an agar slope subcutaneously on six separate occasions.

Summary.

1. The *Micrococcus melitensis* survived for 28 days in natural dust collected from the Strada Mercanti, Malta.

2. The *Micrococcus melitensis* survived for 28 days in the natural building dust found in the streets of Valletta, Malta.

3. A sub-culture from a *Micrococcus melitensis*, which had assumed a saprophytic existence for several weeks in water, did not appear to live longer in soil than a culture freshly isolated from the body of a Mediterranean Fever patient.

4. The *Micrococcus melitensis* survived for 20 days in an unsterilised, manured garden soil.

5. It has not been found possible to infect monkeys with dust polluted with urine from Mediterranean Fever patients and then, thoroughly dried. Goats, however, can be infected in this manner.

V. CONTACT EXPERIMENTS.

By Major W. H. HORROCKS, R.A.M.C.

(Received December 16, 1905.)

In the first report of the Mediterranean Fever Commission a description was given of an experiment in which a healthy monkey living between two infected monkeys became infected with the *Micrococcus melitensis*.

The infection might have occurred in the following ways, *i.e.*, (a) by direct skin contact; (b) by placing in its mouth paws fouled with urine excreted by its neighbours; (c) by means of mosquitoes; (d) by ecto-parasites passing from the skin of the infected monkeys to the healthy monkey.

Experiments were devised in order to ascertain which of the above possible causes had produced the infection. In the first experiment a healthy monkey was placed in a box next to infected monkeys, and the boxes were surrounded by mosquito-proof netting. The monkeys were in intimate contact, and the boxes were frequently changed, the healthy monkey being allowed to live for a few days at a time in the box previously occupied by the infected monkeys. In this experiment the infection might have been carried by skin, urine, or ecto-parasites.

In the second experiment the healthy and the infected monkeys were placed in a small cage covered with coarse wire and divided into two compartments by netting with very large meshes; the netting was fastened to a wooden ledge so arranged that fluid could not pass from one cage to the other. The compartments were of such a shape that when the monkeys were sitting in the cage back to back the skins were in intimate contact, and the monkeys could not turn round. As an additional precaution the arms, legs, and buttocks were placed in mackintosh bags. Infection under these conditions might be carried by both skin and ecto-parasites.

In the third experiment a small mosquito-proof hut was divided into two compartments by fine wire netting fastened below to a board fixed to the terrace by cement. The conveyance of infection by urine, skin, and mosquitos was thus excluded, and only ecto-parasites, which would easily pass through the wire netting, could have operated in conveying the *Micrococcus melitensis* to the healthy animal.

The details of the experiments are as follows:—

Experiment I.—Monkey No. 91 and Monkey No. 95 were inoculated with the *Micrococcus melitensis*, subcutaneously, to act as infecting agents. Monkey No. 91 suffered from a long wave of fever, its temperature rose to 105° on May 29, and remained so with a few intermissions until June 19, when it slowly fell to normal. Its blood

serum diluted 1/500 caused immediate agglutination of the *Micrococcus melitensis*. Monkey No. 95 had a wave of fever lasting from June 20 to June 27, and the blood serum diluted 1/100 reacted with the *Micrococcus melitensis*. The monkey died on July 21, and the specific microbe was recovered from the spleen, bile, mesenteric, femoral and axillary glands.

A wooden framework, covered with mosquito-proof netting, having been erected on the terrace of the Public Health Department, three boxes were placed inside it. On May 25 Monkey No. 92 and Monkey No. 91 were placed in contiguous boxes. The blood serum of No. 92 was examined, but no reaction was obtained.

June 13. Blood of Monkey No. 92 was examined, but no reaction was obtained.

June 14. Monkey No. 92 was found occupying the box of Monkey No. 91.

June 19. The blood of Monkey No. 92 was examined, but without any reaction.

June 24. The blood of Monkey No. 92 was again examined, with a similar result. Monkey No. 95 was put in a box, and the box of Monkey No. 92 was placed between those of Monkeys Nos. 91 and 95.

July 2, July 9, July 16, July 23, and August 9. The blood of Monkey No. 92 was examined, but no reaction appeared.

August 20. The blood serum of Monkey No. 92, diluted 1/10, was found to agglutinate the *Micrococcus melitensis* at once, the reaction being visible to the naked eye.

August 25. Two cubic centimetres of blood were removed from a saphenous vein and planted out in broth-tubes; after seven days' incubation at 37° C. no growth was obtained.

August 31. The blood serum, diluted 1/100, caused immediate clumping of the *Micrococcus melitensis*.

September 11. One cubic centimetre of blood was removed from a saphenous vein and planted out in broth-tubes; after seven days' incubation at 37° C. no growth was obtained.

September 13. The blood reaction was found, as recorded, on August 31.

October 4. The monkey was killed with chloroform. At the *post-mortem* examination the body appeared fairly nourished, and the spleen and glands were not enlarged. Cultures were made from the spleen, liver, kidneys, bile, heart's blood, mesenteric, femoral, and axillary glands. The *Micrococcus melitensis* was recovered from the glands, but the cultures made from the other organs appeared sterile.

Remarks.—The monkey had no rise of temperature during the experiment, and save for a slight loss of flesh appeared perfectly well. The boxes of the monkeys and the floor of the enclosure were

cleansed as little as possible, so as to permit of the infection by urine as well as by the skin and by ecto-parasites. The presence of the *Micrococcus melitensis* in the glands showed that an infection had taken place, and that it was produced by the urine is proved by the results of Experiments II and III.

Experiment II.—A small narrow box covered at the sides with fine wire netting, and having a wooden bottom, was divided into two compartments, each just large enough to hold a monkey in the sitting position, by means of very coarse wire netting. A movable wire floor was sloped from the centre to the sides of each compartment, so that any fluid deposited on it would run off through the wire meshes into the space beneath. By this arrangement the possibility of urine passing from one cage to the other was completely obviated.

Monkey No. 91 was used as the infecting agent, and Monkey No. 88 as the contact.

Monkey No. 88 was kept under observation for 14 days, and its blood repeatedly examined before the experiment was commenced.

On June 8 the monkeys were placed in the cage back to back. The open wire netting of the partition permitted the backs to be in intimate contact, and was merely used to steady the monkeys and prevent them turning round. The arms and legs and buttocks of each monkey were placed in waterproof bags so as to prevent dried urine being conveyed by the infected to the healthy monkey, should the former by any chance manage to turn round in the cage and pass its paws through the loose meshes of the partition. As soon as the monkeys were placed *in situ* the whole cage was covered with mosquito netting.

From June 8 to June 23 Monkey No. 91 and Monkey No. 88 were placed in contact daily for four hours. From June 24 to July 4 Monkey No. 95 was used as the infecting agent. The blood of Monkey No. 88 was examined on June 13, June 18, June 24, and July 2, but no signs of a reaction with *Micrococcus melitensis* were ever seen.

On July 5 Monkey No. 88 was seized with an acute attack of diarrhoea, and he died on July 12.

At the *post-mortem* examination all the organs appeared healthy. Cultures were made from the organs in the usual manner, but all proved sterile.

Remarks.—Infection by urine being excluded, this experiment seems to show that neither ecto-parasites nor intimate skin contact participate in the conveyance of infection from diseased to healthy monkeys.

Experiment III.—The object of this experiment was to ascertain whether ecto-parasites alone could convey infection from a diseased to a healthy monkey.

A small mosquito-proof hut, erected on the terrace of the Public Health Department, was divided into two compartments by strong wire netting, having a meshwork so fine that only small ecto-parasites such as fleas and bugs could pass through it. The wire netting was fastened below to a deal board a foot high and fixed by cement to the floor of the hut.

Monkeys Nos. 110, 92, and 101 were used as the infecting agents. Monkey No. 23, the healthy animal, was kept under observation for a fortnight before the experiment was commenced. Its blood was repeatedly tested, but no reaction with the *Micrococcus melitensis* was observed.

On September 5 Monkey No. 110, which had been injected subcutaneously with the *Micrococcus melitensis* specially for this experiment, was placed in one compartment of the hut, and Monkey No. 23 in the other, the chains of both animals being so arranged that when at full length they could not touch the wire partition. On September 10 Monkey No. 110 showed no sign of fever, and only a very feeble blood reaction. Monkey No. 92 was then placed in its compartment, to act as the infecting agent. On October 5 Monkey No. 92 was removed, and Monkey No. 101, then at the height of a wave of fever, was placed in the infected compartment. The blood of Monkey No. 23 was examined on September 14, 20, and 27, and on October 13 no signs of a reaction with *Micrococcus melitensis* could be detected.

The experiment was continued until November 12, Monkey No. 101 passing through a secondary wave of fever. The blood of Monkey No. 23 was repeatedly examined during November, but no signs of a reaction were observed. The monkey is still alive and well.

Remarks.—Ecto-parasites alone do not appear able to convey infection from a diseased to a healthy monkey.

As the experiments had failed to show that ecto-parasites and direct skin contact play any part in the infection of healthy monkeys living in intimate contact with diseased monkeys, and that the infection was probably caused by the absorption of urine containing the *Micrococcus melitensis*, it appeared desirable to ascertain by direct experiment whether a monkey could be infected by feeding with the urine excreted by Mediterranean Fever patients. Accordingly Monkey No. 119 was fed on urine added to dust and potato, etc.; the details of the experiment were as follows:—

Experiment IV.—Monkey No. 119 was kept under observation in a mosquito-proof chamber for several days before the experiment was commenced. On August 1 the blood was examined, but the serum gave no reaction. The monkey was then fed on fine sterilised dust moistened with urine from a case of Mediterranean Fever containing large numbers of the *Micrococcus melitensis*. The urine dust, thoroughly mixed with potato, was on this occasion readily eaten. On August 2,

3, and 4 the feeding was continued, but it was noticed that after the first feeding the monkey ate very little of the infected potato. On August 7, 8, and 9 attempts were made to get the monkey to drink the urine in its natural state, but with poor success. On August 10 a few cubic centimetres of urine were rubbed up with a considerable quantity of boiled potato, and, as the monkey ate the mixture readily, this method of feeding was continued daily until the 19th, after which date it was fed every other day until the 29th.

The blood was examined on August 11 and 12, but the serum gave no reaction. On August 28, however, slight clumping of the *Micrococcus melitensis* was caused by the serum, diluted 1/10. On August 30 the reaction was quite distinct, the serum being diluted 1/10. On September 5 the blood serum, diluted 1/100, caused instantaneous clumping of the *Micrococcus melitensis*. On September 15 the monkey was killed with chloroform.

At the *post-mortem* examination the spleen was found large, soft, and friable. The axillary, femoral, and mesenteric glands were also found enlarged.

Cultures were made from the spleen, liver, kidneys, bile, heart's blood, axillary, femoral, and mesenteric glands. The *Micrococcus melitensis* was recovered from the axillary, femoral, and mesenteric glands, the plates being crowded with colonies. It was also isolated from the spleen, but only a few colonies were found on the agar slopes. The remaining cultures proved sterile.

Remarks.—The monkey became infected about 28 days after the feeding was commenced. There was no rise of temperature during the experiment.

Summary.

1. So far as the experiments go it appears that infection cannot be conveyed from infected to healthy monkeys by skin contact alone, all other sources of infection being excluded.

2. That infection cannot be conveyed from infected to healthy monkeys by ecto-parasites alone.

3. That when healthy monkeys living in intimate contact with diseased monkeys, under mosquito-proof conditions, become infected, the infection is due to the absorption of the *Micrococcus melitensis* excreted in the urine of the diseased monkeys.

VI. GOATS AS A MEANS OF PROPAGATION OF MEDITERRANEAN FEVER.

By Major W. H. HORROCKS, R.A.M.C., and Captain J. C. KENNEDY, R.A.M.C.

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(PLATE I.)

In Part III of the Reports of the Commission a preliminary note was published on this subject, in which it was shown that goats in Malta suffer from Mediterranean Fever, and excrete the *Micrococcus melitensis* in their milk and urine. The further study of this subject may be divided into the following parts:—

- I. Examination of goats living in pens (a) in the immediate neighbourhood of Valletta and Sliema, and (b) in the more remote parts of the Island.
 - II. Experiments made to determine the possibility of infecting animals by feeding them on milk-cultures and infected milk.
 - III. Experiments to determine the mode in which the goats themselves become infected.
 - IV. Experiments to determine whether it is possible to destroy the *Micrococcus melitensis* by Pasteurisation of the infected milk.
- I. EXAMINATION OF GOATS LIVING IN PENS (a) IN THE IMMEDIATE NEIGHBOURHOOD OF VALLETTA AND SLIEMA, AND (b) IN THE MORE REMOTE PARTS OF THE ISLAND.

(a) Examination of Goats Supplying Milk to Forrest Hospital.

This herd consisted of 15 goats, and five reacted to the *Micrococcus melitensis*. The serum of Goat No. 37 reacted in a dilution of 1/60, but the sera of Goats Nos. 38, 39, 43, and 48 only reacted in a dilution of 1/20.

Goat No. 37.—This goat was in good health, and its udders were full of milk. On July 4, 5, and 6 the plates made with the deposit from 2 c.c. of the milk were found densely crowded with small colonies of the *Micrococcus melitensis*. The milk was again examined on August 12, September 6, and October 8, and on each of these occasions the plates were found densely crowded with colonies of the microbe. The physical characters of the milk appeared perfectly normal at each examination.

Goat No. 38.—The milk from this goat was centrifugalised, and the deposit plated on July 4, 5, 6, 7, 8, 10, 14, 17, 18, 25, August 19, September 6, and October 8. The *Micrococcus melitensis* was never recovered.

Goat No. 48.—The milk from this goat was examined on the same dates as No. 38. The *Micrococcus melitensis* was not isolated.

Goat No. 43.—The milk from this goat was also examined on the same dates as No. 38. The *Micrococcus melitensis* was not recovered.

Remarks.—The examination of Goat No. 37 shows that the *Micrococcus melitensis* may be excreted steadily in milk for three months without any change occurring in its physical characters. It was thought that Goats Nos. 38, 43, and 48 might be in an earlier stage of the disease than No. 37, and that if they were kept under observation the *Micrococcus melitensis* might eventually appear in the milk. Though the goats were kept under examination for three months, the specific *Micrococcus melitensis* was never recovered from the milk.

Examination of a Small Herd Supplying Milk to Valletta Hospital.

This herd consisted of 13 goats, and four reacted to the *Micrococcus melitensis*.

Goat No. 30.—The serum of this goat reacted only in a dilution of 1/20. The microbe was recovered from the milk from July 1 to July 6, but after this it disappeared.

Goats Nos. 27 and 32.—The milk from these goats was examined during June, July, August, and September, but no signs of the *Micrococcus melitensis* were detected. The serum of Goat No. 27 reacted in a dilution of 1/100, and that of No. 32 in a dilution of 1/60.

Remarks.—The *Micrococcus melitensis* was not isolated from the milk of the two goats which judged by the serum reaction would have been considered the most severely infected. In the case of Goat No. 30 the excretion of the parasite continued only for one week, though the secretion of the milk was maintained in good quantity and quality for three months.

Examination of a Small Herd Supplying Milk to Valletta.

This herd consisted of 25 goats, and 17 showed a blood reaction.

Goat No. 50.—Plates made from the milk of this animal were found densely crowded with colonies of the *Micrococcus melitensis*.

Goat No. 52.—During the first week of July the milk of this goat was found to be markedly infected, but examinations made in August, September, and October failed to show any signs of the *Micrococcus melitensis*, though the quantity and quality of the milk continued good.

Goat No. 68.—The milk of this goat contained a comparatively small quantity of the *Micrococcus melitensis*, only 100 colonies being found in the plates made with the deposit from 1 c.c.

Remarks.—The severity of the infection of these goats, judged by the serum reaction, should have been the same, and as the animals were in full milk, the excretion of the microbe might have been expected to occur to the same extent in all the animals. Such was

not the case, and judged by the excretion in the milk it appeared that Goat No. 68 was only infected to a small extent.

Examination of a Small Herd Supplying Milk to Sliema.

Two goats were bought from this herd and placed in the Lazaretto.

Goat No. 15.—The milk of this animal contained large quantities of the *Micrococcus melitensis* during the first week of July, but examinations made in August, September, and October failed to demonstrate the presence of the specific *Micrococcus melitensis*. The quantity and quality of the milk, however, continued good.

Goat No. 16.—The milk of this goat was examined during July, August, September, and October, but no signs of *Micrococcus melitensis* were detected. The blood serum, diluted 1/60, caused immediate agglutination of the microbe when the animal was bought in July, and examinations made at later dates showed that the blood reaction was unchanged.

Remarks.—It appears from the results obtained in the case of Goat No. 16 that an animal may have a marked blood reaction lasting for four months, and yet never excrete the *Micrococcus melitensis* in its milk.

Examination of Goats at Hamrun.

These goats supply a large portion of the milk consumed in Valletta.

The following herds were examined :—

Herd No. 1.—This consisted of 46 goats, and 26 reacted to the *Micrococcus melitensis*, i.e., 6 in a dilution of 1/100, 4 in a dilution of 1/60, 2 in a dilution of 1/40, 9 in a dilution of 1/20, and 5 in a dilution of 1/10. Only one of these goats showed an excretion of *Micrococcus melitensis* in the milk, and the blood serum reacted in a dilution of 1/100.

Herd No. 2.—There were 30 goats in this herd; the *Micrococcus melitensis* was only found in the milk of one goat. The blood could not be examined, as the owner of the goats refused to allow a specimen to be taken.

Herd No. 3.—There were 26 goats in this herd. The *Micrococcus melitensis* was not isolated from the milk of any of them.

Herd No. 4.—There were 45 goats in this herd. All proved to be quite healthy. No signs of the *Micrococcus melitensis* could be discovered in the milk.

Herd No. 5.—This herd consisted of 32 goats. The milk from five of them was found to contain the *Micrococcus melitensis* in large quantity.

Remarks.—Only 3·3 per cent. of the goats examined contained the *Micrococcus melitensis* in the milk.

Examination of Goats at Pietà.

These goats supplied milk to Valletta; there were 32 animals in the herd and 24 reacted to the *Micrococcus melitensis*, i.e., 13 in a dilution of 1/100, 1 in a dilution of 1/60, 2 in a dilution of 1/40, 4 in a dilution of 1/20 and 4 in a dilution of 1/10. The large number of goats with a high serum reaction was remarkable. Only six goats were found excreting the *Micrococcus melitensis* in the milk, and the sera of all of these reacted in a dilution of 1/100.

Remarks.—About 18·7 per cent. of the goats in this herd were found excreting the *Micrococcus melitensis* in the milk.

Examination of Goats at Paolo.

These goats supplied milk to Paolo and parts of Cospicua and Senglea. There were 24 goats in the herd, and 17 reacted to the *Micrococcus melitensis*. Only three goats were found excreting the microbe in the milk; the blood of two of the goats reacted in a dilution of 1/100 and the third in a dilution of 1/60. Three goats having a blood reaction of 1/100 showed no signs of the microbe in the milk.

Remarks.—About 12·5 per cent. of the goats in this herd were found excreting the *Micrococcus melitensis* in the milk, though judged by the blood reaction some 70 per cent. were infected.

Examination of Goats at Attard.

There were 19 goats in this herd which supplied milk to Attard. None of the goats reacted to the *Micrococcus melitensis*, and the milk of all of them was quite free from infection.

Examination of Goats at Citta Vecchia.

These goats supplied the Military Hospital; there were 15 animals in the herd, and 11 were found to react with the *Micrococcus melitensis*. The milk of five goats was found to contain the specific *Micrococcus melitensis*, and of these the blood sera of three reacted in a dilution of 1/100, the serum of the fourth in a dilution of 1/60, and the serum of the fifth in a dilution of 1/40.

Remarks.—About 33 per cent. of the goats supplying the Military Hospital were found excreting the *Micrococcus melitensis* in the milk.

Examination of Goats at Zeitung.

These goats supplied the three cities. There were 93 animals in three herds. The milk of only one goat was found to contain the *Micrococcus melitensis*.



Milk from a goat in a herd at Zabbar. 10 c.c. centrifugalised and three drops of the deposit spread on a glucose-nutrose-litmus-agar plate. Incubated 4 days at 37° C.

From photo. by Staff-Sergeant Rossiter, R.A.M.C.

Examination of Goats at Zabbar. (Plate 1.)

Four herds were examined. There were 44 goats in the first herd, and the *Micrococcus melitensis* was found in the milk of four of them. In the second herd, containing 28 goats, no signs of the microbe could be found in the milk of any of the animals. In the third herd there were 41 goats; the *Micrococcus melitensis* was found in the milk of only one animal. In the fourth herd, consisting of 19 goats, the milk of one was found to contain the *Micrococcus melitensis*.

Remarks.—About 4·5 per cent. of the goats were found excreting the *Micrococcus melitensis* in the milk.

Examination of Selected Goats at Balzan.

The goats were first subjected to the milk test, and 21 of them showed a tendency to agglutinate the *Micrococcus melitensis*. The milk from these animals was carefully "plated," but the microbe was only recovered from the milk of six of them.

Remarks.—About 29 per cent. of the goats selected by the milk agglutination test were found excreting the *Micrococcus melitensis* in the milk.

Examination of Selected Goats at Casal Lia.

These goats were also subjected to the milk test, and 13 appeared to be infected. The *Micrococcus melitensis*, however, was only found in the milk of four of them.

Remarks.—About 30 per cent. of the selected goats were found excreting the *Micrococcus melitensis* in the milk. The percentage was practically the same as that obtained in the case of the goats selected at Balzan.

Examination of Selected Goats at Zabbar.

Five appeared to be infected, judged by the milk test, and the *Micrococcus melitensis* was found in the milk of two of them.

Remarks.—About 40 per cent. of the selected goats appeared to contain the *Micrococcus melitensis* in the milk, but the figures are too small to be of any practical value.

Examination of Goats at Melleha.

There were 91 goats in the herds, and 16 showed a reaction with the *Micrococcus melitensis*; the specific microbe was not found in the milk of any of them.

Examination of Goats at the Lunatic Asylum.

There were 31 goats in this herd, which was kept in the Asylum grounds. The goats were not allowed to graze in the public streets. A careful examination of the milk, by means of the agglutination test,

was made, but no reaction was obtained. Ten cubic centimetres of milk from each animal was then centrifugalised and the deposit "plated;" no signs of the *Micrococcus melitensis* were observed in any of the plates.

The following table gives the degree of reaction obtained in each of the infected goats, worked out to a dilution of 1/100.

Each goat is designated by a number, which is placed in the column corresponding to the highest dilution in which its blood reacted:—

		Dilutions.						Total.
		1/10.	1/20.	1/40.	1/60.	1/80.	1/100.	
Herd	I	2 80	74 78	1 75			83 .	7
"	II						5	1
"	III							0
"	IV		22 30		32		27	4
"	V		38 39 43 43		37			5
"	VI	51 59 62 63 65 73	54 66 67 70		64		50 52 55 60 68 71	17
"	VII	97 109 117 120 123	88 93 96 106 110 112 113 125 128	85 90	103 111 119 126		99 103 105 121 122 129	26
"	VIII	137 140 144 148	134 135 141 149	131 157	147		132 133 136 138 145 151 153 154 155 156 152 160 161	21

	Dilutions.						
	1/10.	1/20.	1/40.	1/60.	1/80.	1/100.	Total.
Herd IX	167 169 185 200 208 215 232 234 242 247	166 174 202 231	163	191			22
„ X	253 255						2
„ XI	281	283	268 273 277 282	261 266 271 274 279	278	260 263 264 270 280	17
„ XII	288 294					302	3
„ XIII	307 316	313 318	311	310	326	309 314 315 321	11
„ XIV	3	7 8 9				1	5
Total number of goats	35	35	12	15	2	39	138

Blood Examination of Goats from various parts of Malta.

Herds and number of goats.	Address.	Supply milk to	Total number tested.	Number which gave a definite blood reaction.	Per- centage of reactions.
I Nos. 1 to 4 and 74 to 83	Casal Tarshiel, near Casal Paolo	Cottonera Hos- pital, Zabbar Gate, and near- lying part of town	14	7	50.0
II Nos. 5 to 17	Zabbar	" "	13	1	7.69
III Nos. 18 to 20	Hamrum	Valletta	3	0	0.0
IV Nos. 21 to 33	Casal Curmi	Valletta Hospital and town	13	4	30.6
V Nos. 34 to 48	St. George's Bay	Forrest Hospital.	15	5	33.3
VI Nos. 49 to 73	St. John's Ditch	Valletta	25	17	68.0
VII Nos. 84 to 129	Hamrun	"	46	26	56.5
VIII Nos. 130 to 161	Pieta	"	32	24	75.0
IX Nos. 162 to 252	Melleiha village	Melleiha Camp, Ghain Tuffeiha, Melleiha vil- lage, and 1 mile radius	91	16	17.6
X Nos. 253 to 259	Birchircara	Birchircara vil- lage	7	2	28.5
XI Nos. 260 to 283	Paolo	Paola and near- lying parts of Cospicua and Senglea	24	17	70.8
XII Nos. 284 to 306	Ghashak	Cospicua, Sen- glea, and Cot- tonera	23	3	13.0
XIII Nos. 307 to 330	Citta Vecchia ...	Hospital	15	11	73.3
XIV Private herd Nos. 1 to 10	Sliema	A few private houses, Sliema	10	5	50.0

Total number of goats examined 331

Number which gave a reaction 138

Percentage of goats found infected = 41.69

Examination of Goats' Milk for Agglutinative Reaction to Micrococcus melitensis (Zammit's Test).

Two methods were tried :—

1. Sedimentation in Tubes.—The milk was diluted with four times the amount of emulsion of *Micrococcus melitensis* and left standing for 12 hours in sedimentation tubes. This method was found to be unreliable, as the sediment often consisted of fat and debris, and always required to be submitted to microscopic examination.

2. Agglutination under the Microscope.—Equal parts of milk and emulsion were placed on a slide and allowed to stand for 12 hours in a moist chamber. At the end of this time the fatty part of the milk had collected in the centre and the surface of the drop, leaving the edges and the bottom clear. The clear part was then examined under 1/6 inch lens for clumping of *Micrococcus melitensis*. It should be noted that the milk was prevented from turning sour by adding one drop of 40 per cent. of formalin to 10 c.c. of milk.

The second method was found much the more certain, and, after a trial of 57 samples of milk examined in both ways, was adopted in preference to the first. Samples of the milk of 57 goats whose blood had been tested were examined in both these ways. The examination of the blood showed a positive agglutinative reaction to *Micrococcus melitensis* in 41. The milk gave a positive reaction—(1) by sedimentation in 17, (2) by microscope in 27.

In all, the reaction of 115 samples of milk was examined, and a positive reaction was obtained in 47. All these samples were more or less selected, and cannot be taken as a fair average; of these, 86 were examined for serum reaction, with the following result :—

Blood positive, milk positive	42 = 48·8 per cent.
Blood positive, milk negative	16 = 18·6 „
Blood negative, milk negative.....	28
	—
	86

∴ Milk gave a reaction in only 72·4 per cent. of those giving a serum reaction.

The numbers of the 16 goats (blood positive and milk negative) mentioned above are as follows :—

No. of goat.	Reaction of blood in dilutions.	Whether <i>Micrococcus melitensis</i> was found in milk ?
43	1/10	No.
48	1/20	"
112	1/20	"
119	1/40	"
140	1/10	"
163	1/40	"
174	1/10	"
215	1/10	"
232	1/10	"
281	1/10	"
282	1/40	"
288	1/10	Not examined.
302	1/100	—
313	1/10	No.
316	1/10	"
318	1/20	"

Remarks on the goats whose blood was examined for reaction to Malta Fever.

It was expected that those goats whose blood reacted would have some symptoms of illness, but this was not apparent except in a few instances. A few goats were noticed to have an unusual degree of lassitude and to be off food. In the later stages of the infection, when the milk was beginning to dry up, a short, hacking cough was noticed, and the goats appeared to steadily lose flesh, the coat also became thin. The quantity and quality of the milk seemed in most cases to be unaffected, indeed it was remarked how often the best milkers in the herd were picked out as a result of the blood examination.

The following goats reacted in a dilution of 1/100, and their daily milk production was as follows :—

No. of goat.	Quantity of milk in pints.
132	3
133	3
136	5
138	2
151	6
153	2
154	6
156	4
159	2
160	2½

A good average milk production is from four to five pints a day, so it appears that when the blood reaction is very marked there may be a diminution in the quantity, though the physical characters and chemical composition of the milk remain unchanged.

Pregnancy goes on uninterruptedly in infected goats; a miscarriage was reported only in one instance.

The Relation of the Blood and Milk Agglutination to the Infection of the Milk.

As a rule, if the blood agglutination was over 40 dilutions, the milk reaction was present in 85 per cent.

If the blood agglutination reached only 20 dilutions, the milk reaction was present in 60 per cent., and in 10 dilutions only 30 per cent.

The following are the numbers of some goats where the reaction in the blood did not go beyond a dilution of 1/10, yet the milk reaction was present:—

307, 148, 137, 39, 38, 185.

It is thrown out as a suggestion that the presence of the reaction in the milk may be a better guide to the presence of *Micrococcus melitensis* in the milk than the examination of the blood, especially when a good case can be brought forward, such as Goat No. 4 (Lazaretto).

In this case the milk reaction was present, though the blood reaction was only 1/10, and at the same time *Micrococcus melitensis* was being excreted in the milk in very considerable quantities. However, as the excretion of *Micrococcus melitensis* continued in the milk, the blood agglutination crept up until it reached 1/40.

II. TO TEST THE VIRULENCE OF THE *Micrococcus melitensis* EXCRETED IN GOATS' MILK, AND THE POSSIBILITY OF INFECTING ANIMALS BY FEEDING WITH MILK CULTURES AND INFECTED MILK.

(A) To Test the Virulence of *Micrococcus melitensis* Isolated from Goats' Milk.

Monkey No. 107 was brought from Calcutta and placed on the terrace of the Public Health Department:—

1905—

- July 18. Examined blood; no reaction obtained.
- „ 19. Injected subcutaneously, growth on an agar slope, isolated from the milk of infected goat No. 5.
- „ 25. Examined blood; dilution 1/10 reacted at once, visible to naked eye.
- „ 30. Examined blood; dilution 1/10 reacted at once, dilution 1/20 reaction not complete.

1905—

- July 31. Drew off 2 c.c. of blood from saphenous vein and planted out in broth.
- Aug. 7. Planted out growth in broth on an agar slope, typical growth of *Micrococcus melitensis* resulted.
- „ 20. Examined blood; dilution 1/50 reacted, but clumping not quite complete.
- Sept. 2. Removed 1·5 c.c. of blood from saphenous vein; after seven days' incubation at 30° C. no growth resulted.
- „ 3. Examined blood; dilution 1/100 reacted at once.
- „ 13. Examined blood; reaction as on the 3rd.
- „ 20. Monkey ill and steadily losing flesh.
- „ 26. Monkey dying. Killed with chloroform.

Post-mortem Examination.—Body much emaciated; lymphatic glands not appreciably enlarged; spleen very small; liver mottled.

Cultures were made from spleen, liver, kidneys, heart's blood, bile, mesenteric, femoral, and axillary glands.

The *Micrococcus melitensis* was recovered from the mesenteric glands, all the other organs remained sterile.

(B) *To Determine the Possibility of Infecting Monkeys and Goats by Feeding with Cultures of Micrococcus melitensis Isolated from Milk.*

Experiment I.—Monkey No. 6 was brought from Calcutta and placed in a mosquito-proof chamber in the Lazaretto:—

1905—

- July 30. Examined blood; no reaction obtained.
- Aug. 1. Fed on *Micrococcus melitensis* isolated from milk of Goat No. 99, growth on an agar slope being made into a paste with potato.
- „ 5. Fed as on the 1st, culture of *Micrococcus melitensis* isolated from milk of Goat No. 17 being used.
- „ 8. Fed as on the 5th.
- „ 9. Fed as on the 5th; culture isolated from milk of Goat No. 15 used.
- „ 10. Examined blood; no reaction obtained.
- „ 12. Fed as on the 9th.
- „ 17. Fed as before; culture isolated from milk of Goat No. 5 used.
- „ 21. Examined blood; no reaction obtained.
- „ 22. Fed as on the 17th.
- „ 24. Fed as on the 5th.
- „ 25. Examined blood; no reaction obtained.
- Sept. 1. Examined blood; serum, diluted 1/10, reacted at once, clumps visible with naked eye.

1905—

Sept. 4. Removed 2 c.c. of blood from saphenous vein and planted out in broth tubes. After seven days' incubation at 37° C., no growth obtained.

„ 5. Examined blood; serum, diluted 1/100, reacted at once.

„ 6. Killed the monkey with chloroform.

Post-mortem Examination.—Body well nourished; spleen slightly enlarged; liver congested; femoral and axillary glands markedly enlarged; mesenteric glands slightly enlarged.

Cultures were made from the spleen, liver, kidneys, bile, heart's blood, mesenteric, femoral, and axillary glands.

The *Micrococcus melitensis* was recovered from all the cultures except those made with the bile.

The agar slopes inoculated with the spleen, and the plates, over which sections of the glands had been rubbed, were densely crowded with colonies.

Remarks.—The monkey never suffered from fever, and gained in weight during the experiment. The animal was killed five days after the blood reacted, in the hope of ascertaining whether the mesenteric glands were infected earlier and to a greater extent than the axillary and femoral glands. No difference, however, could be ascertained between the glands; all the organs appeared to be teeming with the *Micrococcus melitensis*. The intestines were carefully examined, and no signs of any abrasion or inflammation in the mucous membrane could be detected. This experiment proves that the *Micrococcus melitensis* can be absorbed through a healthy mucous membrane, and shows that the organs may be extensively infiltrated with the *Micrococcus melitensis* without apparently producing any prejudicial effect on the health of the animal during the first week after absorption.

Experiment II.—Monkey No. 7 was brought from Calcutta and placed in a mosquito-proof chamber in the Lazaretto.

1905—

July 30. Examined blood; no reaction obtained.

Aug. 1. Fed on potato mixed with an agar culture of *Micrococcus melitensis* isolated from infected Goat No. 154.

„ 5. Fed on potato mixed with an agar culture of *Micrococcus melitensis* isolated from infected Goat No. 30.

„ 8. Fed as on the 5th.

„ 9. Fed as on the 5th.

„ 10. Fed as on the 5th. Examined blood; no reaction obtained.

„ 12. Fed as on the 5th, but culture isolated from Goat No. 17 used.

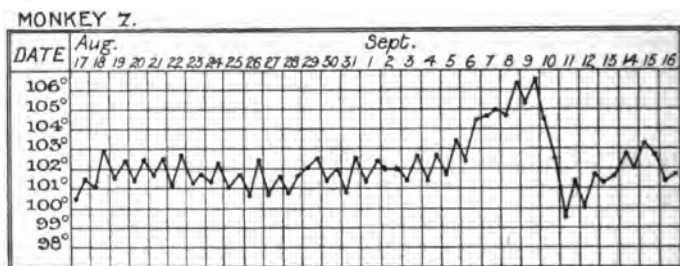
„ 17. Fed as before, agar culture isolated from Goat No. 5 used.

1905—

- Aug. 22. Fed as on the 17th.
 „ 24. Fed as on the 17th, agar culture isolated from Goat No. 60 used.
 „ 25. Examined blood ; no reaction obtained.
 Sept. 1. Examined blood ; no reaction obtained.
 „ 5. Examined blood ; serum, diluted 1/50, reacted at once.
 „ 10. Removed 1 c.c. of blood from saphenous vein and planted in broth-tubes ; after seven days' incubation at 37° C. no growth obtained.
 „ 11. Examined blood ; serum, diluted 1/100, reacted at once.
 „ 15. Removed 2 c.c. of blood from saphenous vein and planted out in broth-tubes ; after seven days' incubation at 37° C. no growth obtained.
 „ 20. Examined blood ; serum, diluted 1/1000, reacted at once.
 „ 24. Killed monkey with chloroform.

Post-mortem Examination.—Body well nourished ; femoral and axillary glands enlarged, mesenteric glands not enlarged ; spleen enlarged, soft and friable ; liver very congested. Cultures were made from the spleen, liver, kidneys, heart's blood, bile, mesenteric, axillary, and femoral glands. The *Micrococcus melitensis* was recovered from the mesenteric, femoral, and axillary glands, the heart's blood, and spleen. The plates inoculated with sections of the glands were densely crowded with colonies, but though 12 agar slopes were inoculated with sections of the spleen, only a few colonies were found on four of them.

The following chart shows the temperature of the monkey during the experiment.



Remarks.—This monkey suffered from a typical wave of fever like Monkey No. 5, fed on infected goat's milk. The distribution of the *Micrococcus melitensis* was very similar in the two monkeys. The interval between the commencement of feeding and the first sign of infection was longer in Monkey No. 7, though it received very much larger doses of the *Micrococcus melitensis*.

Experiment III.—Monkey No. 8 was brought from Calcutta and placed in a mosquito-proof chamber in the Lazaretto.

1905—

- July 30. Examined blood; no reaction obtained.
- Aug. 1. Fed on potato mixed with an agar culture of *Micrococcus melitensis*, isolated from Goat No. 50.
- „ 8, 10, 12 and 18. Fed as on the 1st.
- „ 22. Fed on agar culture, isolated from Goat No. 261.
- „ 24. Fed on agar culture, isolated from Goat No. 138.
- „ 10. Examined blood; no reaction obtained.
- „ 17. Examined blood; no reaction obtained.
- „ 25. Examined blood; slight tendency to a reaction observed.
- Sept. 1. Examined blood; serum, diluted 1/10, reacted at once.
- „ 7. Examined blood; serum, diluted 1/500, gave an immediate reaction.
- „ 8. Removed 2 c.c. of blood from saphenous vein and planted out in broth-tubes; after seven days' incubation at 37° C., no growth obtained.
- „ 14. Removed 2 c.c. of blood and treated as on the 8th, no growth of *Micrococcus melitensis* observed.
- „ 29. Killed the monkey with chloroform.

Post-mortem Examination.—Body well nourished; spleen large and soft; liver and kidneys congested; mesenteric, axillary and femoral glands enlarged.

Cultures were made from the spleen, liver, kidneys, bile, heart's blood, mesenteric, axillary, and femoral glands. A few colonies of the *Micrococcus melitensis* were found in the plates made from the mesenteric, axillary, and femoral glands. All the other cultures proved to be sterile.

Remarks.—This monkey never suffered from fever and put on weight during the experiment.

Experiment IV.—Monkey No. 9, brought from Calcutta and placed in a mosquito-proof chamber in the Lazaretto.

1905—

- July 30. Examined blood; no reaction obtained.
- Aug. 1. Fed on potato mixed with an agar culture of *Micrococcus melitensis* isolated from Goat No. 105.
- „ 5, 8, 9, and 10. Fed as on the 1st.
- „ 12. Fed on agar culture isolated from Goat No. 7.
- „ 17. Fed on agar culture isolated from Goat No. 15.
- „ 22. Fed on agar culture isolated from Goat No. 309.
- „ 24. Fed on agar culture isolated from Goat No. 261.
- „ 10 and 17. Blood examined; no reaction obtained.
- „ 25. Examined blood; distinct reaction after standing for 24 hours.
- Sept. 1. Examined blood; serum diluted 1/10; reacted at once.

1905—

Sept. 7. Removed 2 c.c. of blood from saphenous vein, and planted out in broth-tubes; after seven days' incubation at 37° C. a growth of *Micrococcus melitensis* appeared, which gave all the characteristic tests. Examined blood; serum, diluted 1/1000, gave an immediate reaction.

„ 29. Suffering from fever and losing flesh.

Oct. 2. Very ill, fever still present.

„ 5. Monkey died this morning.

Post-mortem Examination.—Fairly nourished; spleen enlarged; liver congested; mesenteric glands not much enlarged, but congested. A little peritonitis present around the colon.

Cultures were made from the spleen, liver, kidneys, heart's blood, bile, mesenteric, femoral, and axillary glands.

The *Micrococcus melitensis* was recovered from the femoral and axillary glands.

Remarks.—The rise of temperature and subsequent death of this monkey were caused by the peritonitis around the colon. There were no signs of erosion or perforation of the intestine. The peritonitis was probably due to an external injury caused by an iron bracket placed in the wall for the monkey to sit upon.

To Determine the Period of Incubation.

Experiment V.—Monkey No. 19A was placed in a mosquito-proof chamber in the Lazaretto, and kept under observation for a month before the feeding was commenced. Its blood was repeatedly tested, but no reaction was ever obtained. On September 4 it was fed with the growth of an agar slope of *Micrococcus melitensis* isolated from the milk of Goat No. 15. On September 5 the feeding was repeated, but after this date no more culture was given. The blood was examined on September 10, 19, and 23, and on October 5, but no reaction was obtained. On October 7, however, the blood serum, diluted 1/10, caused immediate agglutination of the *Micrococcus melitensis*, the reaction being visible with the naked eye. On October 15 the blood reaction was noticed to be diminishing, as the serum, diluted 1/10, only gave an incomplete reaction with the *Micrococcus melitensis*. On October 16 the monkey was killed with chloroform. At the *post-mortem* examination all the organs appeared healthy. Cultures were made from the spleen, kidneys, liver, bile, heart's blood, and glands. A profuse growth of the *Micrococcus melitensis* was obtained from the spleen and from the femoral and axillary glands. The *Micrococcus melitensis* was also recovered from the heart's blood. The other cultures proved to be sterile.

Remarks.—The monkey never suffered from fever during life, and did not show any signs of ill-health. Although the blood serum had a very feeble agglutinating reaction, the spleen and glands were extensively infiltrated with the *Micrococcus melitensis*.

The period of incubation appeared to be about 32 days.

Feeding Experiment with Milk Cultures.

Goat No. 12 was placed in the Lazaretto and kept under observation for one month before the experiment was commenced.

1905—

- June 26. Examined blood; no reaction obtained.
July 30. Examined blood; no reaction obtained.
„ 31. The growth on one agar slope of *Micrococcus melitensis*, isolated from milk of Goat No. 160, emulsified in water and poured down the throat, no gag being used.
Aug. 5. Fed again as on the 31st.
„ 8. Milk withdrawn from udder, 10 c.c. centrifugalised, and deposit plated on surface of glucose-nutrose-agar plates. No signs of *Micrococcus melitensis* detected.
„ 12. Examined blood; no reaction obtained.
„ 25. Fed on one agar slope of *Micrococcus melitensis*, isolated from milk of Goat No. 5.
„ 29. Fed as on the 25th.
„ 30. Examined blood; no reaction obtained.
Sept. 3. Fed as on the 25th.
„ 5. Fed as on the 25th.
„ 6. Examined blood; no reaction obtained.
„ 9. Fed as on the 25th.
„ 11. Examined blood; no reaction obtained.
„ 13. Fed as on the 25th.
„ 20. Examined blood; no reaction obtained.
„ 26. Examined blood; dilution 1/10, reaction at once.
Oct. 1. Drew off milk, centrifugalised 10 c.c. and plated deposit; no signs of *Micrococcus melitensis* detected.
„ 2. Examined blood; dilutions 1/10, 1/20, and 1/50 gave a complete reaction at once.
„ 6. Removed 5 c.c. of blood from jugular vein and planted in broth-tubes. After seven days' incubation at 37° C. no growth was obtained.
„ 9. Drew off milk, centrifugalised 10 c.c. and plated deposit; no signs of *Micrococcus melitensis* detected.
„ 16, 23, and 30. Milk examined as on the 9th; *Micrococcus melitensis* not recovered.

The examination of the milk and blood was continued once a week until November 30. The blood still reacted in a dilution of 1/40, but no signs of the excretion of the *Micrococcus melitensis* in the milk appeared. The goat is still under observation.

Remarks.—This experiment shows either that the *Micrococcus melitensis* is not always excreted in the milk of goats having a marked blood reaction, or that the excretion is a very late phenomenon.

Experiments to Determine the Possibility of Infecting Monkeys by Feeding them with Goat's Milk Containing the Micrococcus melitensis.

Experiment I.—Monkey No. 5 was brought from Calcutta and placed in a mosquito-proof chamber in the Lazaretto, Malta.

1905—

July 26. Examined blood; serum gave no reaction with the *Micrococcus melitensis*.

„ 27. Fed once a day on milk derived from a small herd of infected goats. There were 11 goats in the herd and the serum from all of them gave a reaction with the *Micrococcus melitensis*. The *Micrococcus melitensis*, however, was only present in the milk of nine of them. A gag was not used; at first the mouth was opened slightly by pressing externally with the thumb and first finger opposite the last molar teeth, but after a few days the monkey learned to lap up milk like a cat.

July 28 to Aug. 6. Feeding continued once a day.

Aug. 4. Feeding continued. Blood examined, serum gave no reaction with the *Micrococcus melitensis*.

„ 5. Feeding continued once a day.

„ 6. Milk given three times a day.

„ 7. Milk given three times a day. Blood examined and no reaction obtained.

„ 8. Milk given three times a day. Blood examined and no reaction obtained.

„ 9. Milk given three times a day. Blood examined and no reaction obtained.

„ 10 to 14. Feeding continued three times a day.

„ 15. Feeding continued. Blood examined; serum showed a slight tendency to react with the *Micrococcus melitensis*.

„ 16. Feeding continued. Blood examined: no distinct reaction obtained.

„ 17 to 25. Feeding continued. The blood was examined on the 21st and 23rd, but no reaction was obtained.

„ 25. Blood examined; complete reaction obtained, serum diluted 1/10. Dilution 1/30 gave no reaction.

1905—

Aug. 27. One cubic centimetre of blood removed from a saphenous vein planted out in broth and incubated at 37° C. Incubation continued for seven days and the broth then planted out on agar slopes. No growth resulted.

Examined blood. Reaction at once, visible to naked eye, serum diluted 1/10. Dilution 1/30 gave a complete reaction after half-an-hour.

„ 28. Removed 1 c.c. of blood from a saphenous vein and treated as on the 27th; no growth resulted.

„ 31. Examined blood; serum diluted 1/100 gave a reaction at once. Dilution 1/200 gave an incomplete reaction after one hour.

Sept. 5. One cubic centimetre of blood removed from a saphenous vein and planted out in broth. After incubation at 37° C. for seven days no growth resulted.

„ 6. Two cubic centimetres of blood removed from a saphenous vein and planted out in broth. After incubation at 37° C. for seven days no growth resulted.

„ 7. Examined blood; serum, diluted 1/100, reacted at once.

„ 11. Examined blood; serum, diluted 1/1000, reacted at once.
Killed the monkey with chloroform.

Post-mortem Examination.—Body well nourished; axillary, femoral, and mesenteric glands enlarged; spleen very large and soft and friable in consistence; liver enlarged and congested; kidneys congested; intestines congested in patches. Cultures were made as follows:—

Spleen.—Planted out on agar slopes (12) and in broth tubes.

Liver.—Planted out on agar slopes (12) and in broth tubes.

Kidney.—Planted out on agar slopes (12) and in broth tubes.

Heart's Blood.—Planted out in broth tubes.

Bile.—Planted out on the surface of glucose-nutrose-agar plates.

Urine.—Planted out on the surface of glucose-nutrose-agar plates.

Axillary Glands.—Sections made and the surfaces rubbed over glucose-nutrose-agar plates.

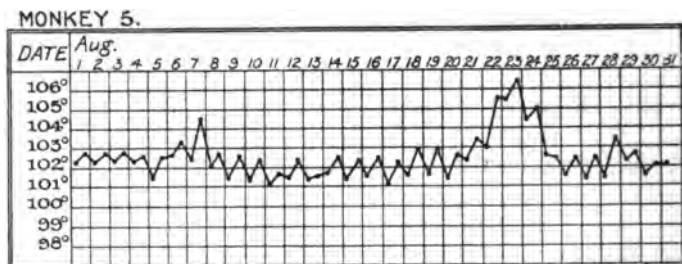
Femoral Glands.—Sections made and the surfaces rubbed over glucose-nutrose-agar plates.

Mesenteric Glands.—Sections made and the surfaces rubbed over glucose-nutrose-agar plates.

After four days' incubation at 37° C., all the plates made from the glands were found densely crowded with colonies of the *Micrococcus melitensis*. Out of the 12 agar slopes inoculated with the spleen only one showed the microbe, and on this slope only four colonies were counted. The cultures made from the liver, kidney and heart's blood

remained sterile. No sign of the *Micrococcus melitensis* could be detected in the plates made from the bile and urine.

The following temperature chart shows a typical short wave of fever such as is usually seen when a monkey is inoculated with the *Micrococcus melitensis* subcutaneously.



Remarks.—After an interval of about 24 days from the commencement of the feeding the monkey suffered from a typical attack of Mediterranean Fever. The distribution of the *Micrococcus melitensis* in the body was somewhat peculiar, the mesenteric and systemic lymphatic glands being densely crowded with the *Micrococcus melitensis*, while the spleen only showed four colonies.

Experiment II.—Monkey No. 4, brought from Calcutta, was placed in a mosquito-proof chamber in the Lazaretto, Malta.

1905—

July 26. Examined blood; serum gave no reaction with the *Micrococcus melitensis*.

„ 28. Fed on mixed milk obtained from the same goats as were used in Experiment I. The same method of feeding was followed as with Monkey No. 5, and was continued from this date until a distinct blood reaction was obtained.

Aug. 4. Examined blood; no reaction obtained.

„ 13. Examined blood; no reaction obtained.

„ 16. Examined blood; no reaction obtained.

„ 21. Examined blood; no reaction obtained.

„ 28. Blood serum, in dilutions of 1/10 and 1/40, gave a complete reaction with the *Micrococcus melitensis*, visible with the naked eye in five minutes.

„ 31. Removed 0.5 c.c. of blood from a saphenous vein and planted out in broth-tubes. After seven days' incubation at 37° C. no growth was obtained.

Sept. 1. Removed 2 c.c. of blood and planted in broth-tubes. After seven days' incubation at 37° C. no growth was obtained.

1905—

- Sept. 3. Blood serum diluted 1/50 gave an immediate reaction.
 „ 9. Removed 1 c.c. of blood and planted in broth-tubes.
 After seven days' incubation at 37° C. no growth was obtained. Blood serum, diluted 1/500, gave an immediate reaction with the *Micrococcus melitensis*.
 „ 16. Removed 2 c.c. of blood and planted in broth-tubes.
 After seven days' incubation at 37° C. no growth was obtained.
 „ 21. Killed monkey with chloroform.

Post-mortem Examination.—Body well nourished; spleen enlarged, soft and friable in consistence; liver very congested; kidneys congested; axillary and femoral glands enlarged; mesenteric glands not enlarged. Cultures were made as follows:—

Spleen.—Planted out on 12 agar slopes and in broth-tubes.

Liver.—Planted out on 12 agar slopes and in broth-tubes.

Kidney.—Planted out on 12 agar slopes and in broth-tubes.

Heart's Blood.—Planted out in broth.

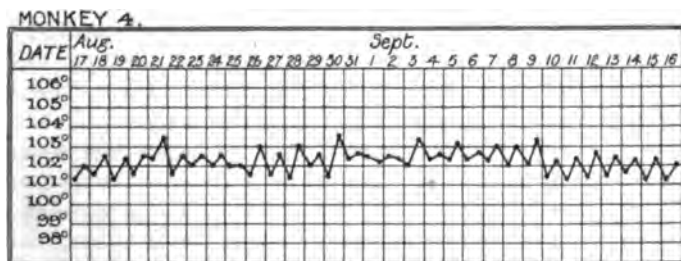
Bile.—Planted out on the surface of glucose-nutrose-agar plates.

Artillery Glands.—Sections made and then rubbed over glucose-nutrose-agar plates.

Femoral Glands.—Sections made and then rubbed over glucose-nutrose-agar plates.

Mesenteric Glands.—Sections made and then rubbed over glucose-nutrose-agar plates.

After four days' incubation at 37° C. only 15 colonies were found on the agar slopes inoculated from the spleen. The liver and kidney cultures were sterile, and no signs of the *Micrococcus melitensis* were observed on the plates made from the bile and axillary glands. Numerous colonies, however, were found in the plates made from the femoral and mesenteric glands. The following chart shows the temperature of the monkey during the experiment:—



Remarks.—After an interval of 33 days from the commencement of feeding this monkey became infected, but there was never any wave of fever as in Experiment I. The distribution of the *Micrococcus*

melitensis in the body was similar to that observed in the first experiment.

Experiment III.—Monkey No. 2 was brought from Calcutta, and placed in a mosquito-proof chamber in the Lazaretto, Malta.

1905—

- July 26. Examined blood; serum gave no reaction with the *Micrococcus melitensis*.
 „ 27. Fed on mixed milk as in Experiments I and II.
 „ 28 to Aug. 3. Feeding continued.
 Aug. 4. Examined blood; no reaction obtained.
 „ 4 to 12. Feeding continued.
 „ 13. Examined blood; no reaction obtained.
 „ 13 to 23. Feeding continued.
 „ 24. Examined blood; no reaction obtained.
 „ 24 to 27. Feeding continued.
 „ 28. Examined blood; no reaction obtained.
 „ 28 to Sept. 2. Feeding continued.
 Sept. 3. Examined blood; no reaction obtained.
 „ 3 to 7. Feeding continued.
 „ 8. Examined blood; no reaction obtained.
 „ 8 to 17. Feeding continued.
 „ 18. Examined blood; no reaction obtained.
 „ 18 to 23. Feeding continued.
 „ 24. Examined blood; no reaction obtained.
 „ 24 to 27. Feeding continued.
 „ 28. Examined blood; no reaction obtained.
 „ 28 to Oct. 2. Feeding continued.
 Oct. 3. Examined blood; no reaction obtained.
 „ 4 to 8. Feeding continued.
 „ 9. Examined blood; a slight reaction obtained after two hours.

From this date the blood was examined weekly, but a definite reaction was not obtained again. The monkey died suddenly on Oct. 18. At the *post-mortem* examination a localised abscess was found round the sigmoid flexure. The organs appeared healthy. Cultures were made from the spleen, glands, and heart's blood. The *Micrococcus melitensis* was isolated from the spleen and glands.

A specimen of blood obtained at the *post-mortem* was tested for agglutination. A dilution of 1/10 gave an incomplete reaction.

Remarks.—During life the monkey suffered from a wave of fever which was attributed to an infection with the *Micrococcus melitensis*, but the *post-mortem* examination showed that the febrile condition might have been due to the abscess round the sigmoid flexure. Although a specific infection undoubtedly occurred, as shown by the isolation of the *Micrococcus melitensis* from the spleen and glands, the blood serum

only once during life caused complete agglutination of the *Micrococcus melitensis*, and that only in a dilution of 1/10.

Experiment IV.—Monkey No. 99 was brought from Calcutta and placed on the terrace of the Public Health Department, Valletta :—
1905—

- June 21. Examined blood ; no reaction obtained.
 „ 26. Examined blood ; no reaction obtained. Fed on milk from infected Goat No. 2.
 „ 27. Fed on milk from infected Goat No. 2.
 July 2. Examined blood ; no reaction obtained.
 „ 9. Examined blood ; no reaction obtained.
 „ 16. Examined blood ; no reaction obtained.
 „ 22. Examined blood ; no reaction obtained.
 „ 23. Fed on mixed milk obtained from infected herds at Pieta and Hamrun.
 „ 24 to 28. Feeding continued.
 „ 29 to Aug. 8. Fed on mixed milk obtained from the infected goats in the Lazaretto.
 Aug. 6. Examined blood ; no reaction obtained.
 „ 15. Examined blood ; no reaction obtained.
 „ 16 to 21. Fed on milk from infected Goat No. 37.
 „ 22. Monkey suffering from diarrhoea. Feeding discontinued.
 „ 23. Examined blood ; no reaction obtained.
 „ 24 to 29. Fed on milk from Goat No. 37.
 „ 30. Examined blood ; slight reaction with serum diluted 1/10.
 Sept. 3. Examined blood ; distinct reaction after half an hour, serum diluted 1/10.
 „ 5. Examined blood ; serum diluted 1/100 reacted at once, and diluted 1/300 reacted in half an hour.
 „ 9. Removed 2 c.c. of blood from a saphenous vein and planted out in broth-tubes. After seven days' incubation at 37° C. no growth obtained.
 „ 17. Removed 2 c.c. of blood and treated as on the 9th ; no growth obtained.
 „ 25. Killed monkey with chloroform.

Post-mortem Examination.—Fairly nourished ; axillary and femoral glands very large, mesenteric glands enlarged ; spleen large, soft and friable ; liver very congested.

Cultures were made as follows :—

Spleen.—Planted out on agar slopes and in broth-tubes.

Liver.—Planted out on agar slopes and in broth-tubes.

Kidney.—Planted out on agar slopes and in broth-tubes.

Heart's Blood.—Planted out in broth-tubes.

Bile.—Planted out on the surface of the glucose-nutrose-agar plates.

Femoral Glands.—Sections made and then rubbed over glucose-nutrose-agar plates.

Axillary Glands.—Sections made and then rubbed over glucose-nutrose-agar plates.

Mesenteric Glands.—Sections made and then rubbed over glucose-nutrose-agar slopes.

After four days' incubation at 37° C. the *Micrococcus melitensis* was recovered from the plates made from the mesenteric glands.

Remarks.—A blood reaction pointing to the infection was not obtained until about 70 days from the commencement of feeding. The monkey never suffered from fever, but the high dilution in which the blood serum reacted and the presence of the *Micrococcus melitensis* in the mesenteric glands showed that a true infection had taken place.

To Determine the Possibility of Infecting a Goat by Feeding on Infected Milk.

Goat No. 8.—This goat had been in the Lazaretto for two months before the experiment was commenced :—
1905—

June 27. Examined blood ; no reaction obtained.

„ 29. Fed on mixed milk obtained from infected goats in the Lazaretto.

July 1. Fed as on the 29th.

„ 8. Examined blood ; no reaction obtained.

„ 13. Removed 10 c.c. of milk from udder, centrifugalised specimen, and plated deposit on glucose-nutrose-agar plates. No signs of the *Micrococcus melitensis* detected.

„ 28. Examined blood ; no reaction obtained.

„ 29 to August 24. Fed three times a day on mixed milk derived from the infected goats in the Lazaretto.

Aug. 4. Examined blood ; no reaction obtained.

„ 13. Examined blood ; no reaction obtained.

„ 22. Examined blood ; no reaction obtained.

Sept. 26. Examined blood ; no reaction obtained.

Oct. 7. Examined blood ; serum diluted 1/10 gave an incomplete reaction.

„ 9. Examined blood ; serum diluted 1/10 gave an immediate reaction, visible with the naked eye. Removed 5 c.c. of blood from the jugular vein and planted out in broth-tubes ; after seven days' incubation at 37° C. no growth obtained. Drew off 10 c.c. milk, centrifugalised specimen, and planted out deposit on glucose-nutrose-agar plates. No signs of *Micrococcus melitensis* detected.

1905—

- Oct. 18. Examined blood ; serum diluted 1/10 gave an incomplete reaction.
- „ 23. Milk centrifuged and deposit plated ; *Micrococcus melitensis* not recovered.
- „ 25. Blood serum diluted 1/10 gave an incomplete reaction.
- „ 30. Milk examined as on the 23rd. *Micrococcus melitensis* not recovered.
- Nov. 30. The *Micrococcus melitensis* has not yet appeared in the milk. The goat is still under observation.

To Test the Possibility of Infecting a Kid by Feeding on Infected Milk.

Kid No. 9.—This kid was under observation for about two months before this experiment was commenced. The blood was repeatedly examined, but no reaction could be obtained with the *Micrococcus melitensis*. It was placed in a room quite apart from the infected goats :—

1905—

- July 13. Examined blood ; no reaction obtained. Commenced feeding with milk obtained from infected goats in the Lazaretto.
- „ 14 to 27. Fed once a day on the infected milk.
- „ 28 to August 28. Fed three times a day on the infected milk.
- July 22. Examined blood ; no reaction obtained.
- „ 30. Examined blood ; no reaction obtained.
- Aug. 7. Examined blood ; no reaction obtained.
- „ 13. Examined blood ; no reaction obtained.
- „ 21. Examined blood ; no reaction obtained.
- „ 27. Examined blood ; no reaction obtained.
- Sept. 5. Examined blood ; no reaction obtained.
- „ 14. Examined blood ; no reaction obtained.
- „ 26. Examined blood ; dilution 1/10 reacted at once, clumps visible with naked eye.
- Oct. 8. Examined blood ; dilution 1/10 gave an immediate reaction, dilution 1/20 “nil.”
- „ 31. Killed the kid and made cultures from the spleen, kidneys, liver, glands, and blood from the inferior vena cava. The *Micrococcus melitensis* was not recovered from any of the organs.

Remarks.—Though a blood reaction was obtained, a true infection did not appear to have taken place. Three other kids fed in the same manner are still under observation.

Feeding with Infected Milk and also with Culture Isolated from Spleen of Man.

Goat No. 4.—This goat was kept under observation for three months before the experiment was commenced. Its blood was repeatedly examined, but never showed the slightest reaction with the *Micrococcus melitensis*. Milk was removed on several occasions, and the deposit obtained by centrifugalisation plated; no signs of the specific micrococcus were observed. On June 27 the goat was fed on infected milk obtained from Goat No. 2. On June 28 mixed milk, obtained from an infected herd, was given. Feeding with this milk was continued until August 16. The blood serum was examined weekly during June, July, and August, but never showed the slightest power of agglutinating the *Micrococcus melitensis*. On September 4 the goat was fed with an emulsion in water of an agar growth of the *Micrococcus melitensis* isolated from the spleen of man. This feeding was continued every other day until the growth of *Micrococcus melitensis* on six agar slopes had been consumed. Examination of the blood for agglutinating reaction, and of the milk for the *Micrococcus melitensis*, was continued weekly. On October 6 the blood serum, diluted 1/10, was found to give an immediate reaction with the *Micrococcus melitensis*. On October 8, 5 c.c. of blood were removed from the jugular vein, and planted out in broth-tubes. After seven days' incubation at 37° C. the broth-tubes were found to be sterile. On October 8 blood was again removed from the jugular vein, but the *Micrococcus melitensis* was not recovered. On October 18 the blood reaction was found to be the same as on the 6th of that month; dilutions above 1/10 failed to give any reaction. On the 23rd, 10 c.c. of the milk were centrifuged, and the deposit plated; the *Micrococcus melitensis* was recovered in small quantity. On October 30 the *Micrococcus melitensis* was found in considerable quantity in the milk. On November 2 the blood serum, diluted 1/20, gave a reaction with the *Micrococcus melitensis*. On November 3 the milk became scanty and thin and serous in character; the microbe could no longer be isolated from it. On November 6 the milk secretion was almost arrested, and on the 13th it had disappeared completely. On November 9 the blood reaction was found to have risen slightly, a dilution of 1/40 causing a complete agglutination of the *Micrococcus melitensis*.

Remarks.—The excretion of the *Micrococcus melitensis* in the milk did not take place until 50 days after the first feeding with the agar-culture derived from the spleen of man, and persisted for only three days.

III. TO DETERMINE THE MODE IN WHICH GOATS BECOME INFECTED.

It appeared possible that goats might become infected—

- (a) By feeding on infected dust.
- (b) By feeding on infected milk.
- (c) By inoculation through the agency of infected mosquitos.
- (d) By inoculation through the agency of infected blood-sucking flies.
- (e) By direct transmission from mother to kid.

(a) To Determine the Possibility of Infecting Goats by Feeding them on Infected Dust.

Goat No. 13.—This goat was in full milk when bought. It was taken to the Lazaretto and placed in a room quite apart from the infected goats. Before the experiment was commenced the blood was repeatedly examined, but no signs of a reaction were observed. On July 13 dust, infected with goat's urine containing the *Micrococcus melitensis* and then dried, was sprinkled over the food. This was done daily until July 22, when dust infected from a case of Mediterranean Fever was used. This dust, dried for 24 hours at room temperature, was sprinkled over the food for a further period of three weeks. The blood was tested weekly for a reaction with the *Micrococcus melitensis*, and on August 3 the blood serum, diluted 1/10, was found to give an instantaneous reaction. The examination of the blood was continued, but the serum reaction never rose above a dilution of 1/10. Five cubic centimetres of blood were removed once a month from the jugular vein, but the *Micrococcus melitensis* was not isolated.

Every week 10 c.c. of the milk were centrifugalised, and the deposit plated. On October 16 the *Micrococcus melitensis* was isolated from the milk. No rise of temperature was ever observed. The goat is still under observation.

Remarks.—This experiment proves that a goat can be infected by feeding on dust infected with urine from Mediterranean Fever patients. The excretion of the *Micrococcus melitensis* in the milk appears to be a late phenomenon, as it was not seen until 74 days after the blood reaction.

Goat No. 14.—This experiment was conducted on the same lines as the one just described. The dust, however, was often slightly moist, instead of being thoroughly dried as in the case of Goat No. 13. The feeding was commenced on July 20, and continued until September 1. The blood was tested weekly for a reaction with the *Micrococcus*

melitensis. On September 3 the blood serum, diluted 1/10, gave a distinct reaction. On October 2 the blood serum, diluted 1/20, caused instantaneous clumping of the *Micrococcus melitensis*, but on October 24 the working dilution was only 1/10. Every week 10 c.c. of the milk were centrifugalised, and the deposit plated, but up to the present the microbe has not appeared in the milk. The goat is still under observation.

Remarks.—As the dust was imperfectly dried, it was thought that Goat No. 14 would have been more seriously affected than Goat No. 13, and it was expected that the *Micrococcus melitensis* would appear in the milk at an earlier date.

(b) *To Determine the Possibility of Infecting Goats and Kids by Feeding them on Infected Goat's Milk.*

It is the custom to feed young kids on milk, and this mode of infection appeared probable, but whether the infection so acquired will persist until the adult stage can only be proved by keeping the animals under observation for a prolonged period.

The histories of Goat No. 8 and Kid No. 9 show that a blood reaction may be acquired in this manner. Goat No. 8 is still under observation. Kid No. 9 was killed, but the *Micrococcus melitensis* was not isolated from its organs, so a true infection did not appear to have taken place in this case.

Five other kids have been fed on infected milk, and are being kept under observation.

(c) *To Determine the Possibility of Infecting Goats through the Agency of Mosquitoes.*

In another section of this report we have shown that *Culex pipiens* and *Stegomyia fasciata* may carry the *Micrococcus melitensis*, consequently it appeared desirable to test by actual experiment whether the microbe could be conveyed from an infected to a healthy goat through their agency.

Culex pipiens, *Stegomyia fasciata*, and *Acartomyia Zammitii* were bred from larvæ. About 50 imagoes of each kind were placed in separate cages and fed on the goats from whose blood Zammit had previously recovered the *Micrococcus melitensis*. The cages were transferred to the healthy goats after periods which varied in each series of experiments. In the first series the interval between the feeding on the infected and healthy goats was 48 hours, in the second series the interval was 72 hours. Forty-eight hours after feeding on the healthy goats the mosquitoes were again transferred to the infected goats. The blood of each healthy goat employed in the experiments was tested twice a week for a reaction with the *Micrococcus melitensis*. The experiments

were continued for two months. It was found that on an average the mosquitoes only lived for about 14 days, so fresh batches had to be employed as the old ones died off.

The healthy goats never showed the slightest sign of a blood reaction with the *Micrococcus melitensis*.

In these experiments the cages could not be fastened to the goats for a longer period than two hours, as the animals struggled violently when kept in one position for a longer time. It was thought that the failure might have been due to an unsuitable selection of the intervals between the various feedings, and to the comparatively short time that the mosquitoes were in actual contact with the animals.

An attempt was now made to imitate the conditions actually occurring in daily life. A small recess in one of the large rooms in the Lazaretto was divided into two compartments, each large enough to hold a goat comfortably, by means of a partition made of coarse wire netting fastened below to a board 3 feet in height. The board was fixed to the floor of the recess by cement, so that urine could not possibly pass from the infected to the healthy goat. The top and front of the recess were made mosquito proof, and a small mosquito-proof door was provided for each compartment. Brackets were fastened on the walls of each compartment out of reach of the goats. Jars containing water full of larvæ in the pupal stage were then placed on the brackets in the compartment containing the infected goat, and jars containing water free from larvæ were placed on the brackets in the compartment for the healthy goat. The wooden portion of the partition prevented the goats coming into actual skin contact, but the large apertures in the wire netting readily permitted the mosquitoes to fly from one compartment to the other. This experiment was continued for a month, fresh water containing larvæ being placed in the compartment containing the infected goat as the imagoes died off. At a later period imagoes were bred out in cages and then let loose in the infected compartment. The blood of the healthy goat was examined twice a week, but it never showed the slightest sign of a reaction with the *Micrococcus melitensis*. The goat is still under observation.

Result.—Up to the present time infection has not been conveyed to healthy goats by means of mosquitoes which had previously fed on infected goats. The failure of the experiments may be due to the *Micrococcus melitensis* being present in the blood of infected goats in too small quantity to be conveyed by mosquitoes. In the feeding experiments already described 5 c.c. of blood were frequently taken from the jugular vein of infected goats, and yet the *Micrococcus melitensis* was never isolated. Zammit succeeded in isolating the *Micrococcus melitensis* from the blood of the infected goats used in the experiments once only, all his further attempts failed.

Knowing how intimately goats live with the Maltese people, it seems probable that, if mosquitoes do convey infection to goats, the infecting microbe is obtained from man and not from the goat.

(d) *To Determine the Possibility of Infecting Goats through the Agency of Blood-sucking Flies.*

Stomoxys calcitrans having been noticed to infest the goats in Malta, it was thought that this fly might act as an infecting agent. Cages containing about 50 of these flies were placed on the infected goats used in the mosquito experiments, and then transferred after varying periods to a healthy goat. It was soon noticed, however, that unless the flies were fed every 48 hours a very heavy mortality took place. Accordingly the interval between the feedings was mainly kept to this period. The flies fed extremely well on the goats, and their bodies were noticed to be distended with blood. The healthy goat's blood was examined twice a week, but no sign of a reaction with the *Micrococcus melitensis* was ever obtained. The experiments were continued for two months, fresh batches of flies being employed as the old ones died off.

Result.—Up to the present infection has not been conveyed to healthy goats by *Stomoxys calcitrans* which had been previously fed on infected goats. The cause of failure may be that suggested in the mosquito experiments. *Stomoxys* will bite man, and it may be that infection is conveyed from man to goat by its means.

The *Micrococcus melitensis* has not yet been isolated from *Stomoxys* fed on goats and monkeys, and on humanitarian grounds it has not been possible to feed the flies, which produce much swelling and irritation, on patients suffering from Mediterranean Fever.

(e) *To Test the Possibility of Direct Transmission of Infection from Mother to Kid.*

When the herd of goats at Pieta was being examined, it was noticed that one of them (No. 19) was pregnant. The goat only gave one pint of milk, which was normal in appearance, and was used for feeding the kids attached to the herd. The blood of the goat was examined, and it was found that the serum, diluted 1/100, caused immediate agglutination of the *Micrococcus melitensis*. The animal was then purchased, and brought to the Lazaretto on July 16. On July 17 10 c.c. of the milk were centrifugalised, and the deposit spread on glucose-litmus-nutrose-agar plates. After four days' incubation at 37° C., all the plates were found densely crowded with colonies of the *Micrococcus melitensis*. The milk was examined weekly until October 23, and on each occasion a rich growth of the microbe was obtained. On August 14 and September 14, 5 c.c. of blood were removed from

the jugular vein and planted out in broth-tubes. On both occasions the tubes were found quite sterile after 10 days' incubation. On October 25 it was noticed that the blood reaction had diminished, a dilution of 1/50 producing complete agglutination, but 1/100 merely produced a few small clumps. On the afternoon of October 25 the goat dropped her kid, which was a male, and quite strong and healthy, it was numbered 19A. On October 27, 5 c.c. of blood were taken from the jugular vein of the goat, but the *Micrococcus melitensis* was not recovered. The milk was examined on the same day, and found to be teeming with the specific *Micrococcus melitensis*. On October 30 and November 3 the milk was examined again, but no traces of the *Micrococcus melitensis* could be discovered. On November 2 the blood reaction was tested again, and it was found that the serum, diluted 1/125, caused complete agglutination, and small clumps were produced by a dilution of 1/200. On November 13 the serum, diluted 1/200, caused a complete reaction. At the end of November the *Micrococcus melitensis* again appeared in the milk.

Kid (19A) dropped by Goat No. 19 on October 25. On October 26 the blood was tested for agglutination, and it was found that the serum, diluted 1/50, caused an immediate complete reaction, whilst a dilution of 1/100 only produced a few small clumps. It was noticed that the blood reaction corresponded exactly to that of the mother on the day the kid was born. On November 4 the blood reaction was found unchanged, but on November 13 a dilution of only 1/20 was found capable of producing a complete agglutination of the *Micrococcus melitensis*. The kid was then killed and cultures were made from the organs. The *Micrococcus melitensis* was not isolated.

Remarks.—This experiment appears to prove that agglutinins are transferred *in utero* from mother to kid. It also shows that pregnancy may progress in a perfectly normal manner when the mother is markedly infected. The suppression of the excretion of *Micrococcus melitensis* in the milk for nearly a month after the birth of the kid is interesting.

IV.—EXPERIMENTS TO DETERMINE WHETHER IT IS POSSIBLE TO DESTROY THE *Micrococcus melitensis* BY PASTEURISATION OF THE INFECTED MILK.

Experiment I.—Milk was drawn from goats 1, 2, 3, 5 and 6 and thoroughly mixed. This mixed milk was selected for experiment as it contained thick ropy masses, and it was thought that these might act as protecting envelopes to the specific micrococci, so that if such a milk were sterilised by Pasteurisation, it might safely be concluded that any ordinary normal looking milk would be equally sterilised by the same operation.

One loopful of the mixed milk was stroked on a series of surface plates to act as a control. The mixed milk was then heated on a water-bath to 68° C. and this temperature was maintained for 10 minutes. The milk was then rapidly cooled and 10 c.c. of the sterilised milk were spread over 20 litmus-nutrose-agar plates.

Results.—After four days' incubation at 37° C. the control plates were found densely crowded with colonies of the *Micrococcus melitensis*; the plates made from the Pasteurised milk remained free from any signs of the specific *Micrococcus melitensis*, even though the incubation was continued for 14 days.

Experiment II.—This served as a control of Experiment I, the same procedure being followed. The *Micrococcus melitensis* appeared in the control plates, but the plates made with the Pasteurised milk again remained perfectly sterile.

Summary.

1. Judged by the serum reaction, 41 per cent. of the goats in Malta are infected.

2. Ten per cent. of the goats supplying milk to various parts of Malta appear to excrete the *Micrococcus melitensis* in the milk.

3. The excretion of the specific microbe may continue steadily for three months without any change occurring in the physical character or chemical composition of the milk, and without the animal exhibiting any signs of ill health.

4. Some infected goats may lose flesh and their coats may become thin; they may also suffer from a short hacking cough. A febrile condition, however, has not been observed.

5. Goats may have a marked blood reaction (1/100), and yet never excrete the *Micrococcus melitensis* in the milk.

6. If the blood serum or milk does not agglutinate the *Micrococcus melitensis*, the specific microbe is not found in the milk.

7. There is no constant relation between the amount of agglutinins in the milk or blood, and the excretion of *Micrococcus melitensis* in the milk; but the higher the dilution of the serum which agglutinates the *Micrococcus melitensis*, the greater is the probability of finding the *Micrococcus melitensis* in the milk.

8. The excretion of the *Micrococcus melitensis* in the milk may be intermittent, appearing for a few days and then disappearing for a week or more.

9. A blood reaction may exist for some weeks before the *Micrococcus melitensis* is excreted in the milk.

10. If blood cannot be obtained the milk reaction with the *Micrococcus melitensis* (Zammit's test) is a good indication of infection.

11. The milk agglutination test is a surer indication of the *Micrococcus melitensis* being excreted in the milk than the serum reaction.

12. Monkeys and goats can be infected by feeding with cultures of *Micrococcus melitensis* isolated from milk, and also by feeding with infected milk itself.

13. The incubation period in feeding experiments appears to vary between three and four weeks.

14. Monkeys infected by feeding sometimes suffer from a typical wave of fever and lose flesh, at other times they show no obvious signs of ill health, and may even gain in weight.

15. When monkeys become infected by feeding with milk, the lymphatic glands always contain far more colonies of the *Micrococcus melitensis* than the spleen. This fact suggests that the specific micrococci contained in the food are carried to the lymphatic glands and there undergo considerable multiplication. It has not yet been proved that the mesenteric glands are always infected at an earlier date than the femoral and axillary glands, but Experiment IV, feeding with milk, shows that this may be the case at times.

16. It has been demonstrated that goats may become infected by feeding on dust polluted with urine from cases of Mediterranean Fever. The excretion of *Micrococcus melitensis* in the milk resulting from such infection, is a late phenomenon, only appearing about 74 days after the blood reaction has developed.

17. It has not been possible yet to convey infection from goat to goat by means of mosquitoes or *Stomoxys calcitrans*. If mosquitoes do carry the infection, it seems more probable that the microbe is transferred from man to goat, than from goat to goat.

18. Agglutinins may be transferred from the mother to the foetus *in utero*. Pregnancy appears to follow a normal course in infected goats.

19. Pasteurisation (68° C. for 10 minutes) destroys the *Micrococcus melitensis* present in infected goat's milk.

VII.—MOSQUITOES AS A MEANS OF DISSEMINATION OF MEDITERRANEAN FEVER.

By Major W. H. HORROCKS, R.A.M.C., and Captain J. C. KENNEDY,
R.A.M.C.

(Received December 16, 1905.)

(PLATE 2.)

Epidemiological inquiries having shown that while the consumption of infected milk may, and probably does, account for much of the Mediterranean Fever amongst the Maltese, yet many cases occur among the military and naval populations in Malta which cannot be attributed to this cause. Accordingly, a study of mosquitoes as possible carriers of the *Micrococcus melitensis* was commenced. The work done may be arranged in three parts :—

Part I.—A study of the species of mosquitoes found in Malta, and their distribution in the island.

Part II.—Examination of the species to determine whether any of them act as carriers of the *Micrococcus melitensis*.

Part III.—Experiments to determine whether any of the species are capable of conveying infection from cases of Mediterranean Fever occurring in man to healthy monkeys, or from infected to healthy monkeys.

PART I.—STUDY OF THE SPECIES OF MOSQUITOES FOUND IN MALTA.

Mosquitoes were caught in the Military Hospital, Valletta ; Military Hospital, Cottonera ; Military Hospital, Citta Vecchia ; Military Hospital, Imtarfa ; Fort Chambray, Gozo ; Military Hospital, Forrest Hill ; Floriana Barracks ; Civil Hospital, Floriana ; Naval Hospital, Bighi ; Fort Ricasoli ; Barracks, Lower St. Elmo ; Sliema ; Birchircara ; Barracks Imtarfa ; Barracks Cottonera. The following species were recognised :—

Culex pipiens.

Culex fatigans.

Culex spathipalpis.

Stegomyia fasciata.

Acartomyia Zammitii.

All the species, except *Culex spathipalpis*, were found at times full of blood. *Acartomyia Zammitii* and *Stegomyia fasciata* attack human beings both day and night. Whilst *Culex pipiens* and *Culex fatigans* only become troublesome at night.

The distribution of the mosquitoes found was chiefly as follows:—

Military Hospital, Valletta—

Culex pipiens.

Culex fatigans (very few).

Acartomyia Zammitii } These are very rare after
Stegomyia fasciata } the end of September.

Military Hospital, Cottonera—

Culex pipiens.

Culex fatigans.

Culex spathipalpis.

Acartomyia Zammitii.

Stegomyia fasciata.

Military Hospital, Citta Vecchia—

Culex pipiens.

Culex spathipalpis } Comparatively rare.
Stegomyia fasciata }

Military Hospital, Imtarfa—

Culex pipiens (very common).

Culex spathipalpis (rare).

Fort Chambray, Gozo—

Culex pipiens.

Culex fatigans.

Acartomyia Zammitii.

Floriana Barracks—

Culex pipiens.

Civil Hospital, Floriana—

Culex pipiens.

Culex fatigans.

Stegomyia fasciata.

Ricasoli Fort—

Culex fatigans.

Culex pipiens.

Acartomyia Zammitii.

Stegomyia fasciata.

Lower St. Elmo Barracks—

Culex pipiens.

Acartomyia Zammitii.

Stegomyia fasciata.

The *Acartomyia Zammitii* breeds in the salt pools found close to the sea, and it appeared important to determine whether this mosquito invades the small towns and barracks in the interior of the island. More than 600 mosquitoes were collected from Citta Vecchia and Imtarfa, and yet *Acartomyia Zammitii* was never found once among them. Severe outbreaks of Mediterranean Fever having occurred at

both these places, the distribution indicates that, even if Mediterranean Fever be a mosquito-borne disease, the probabilities are against *Acartomyia Zammitii* being an important infecting agent.

The seasonal prevalence of the mosquitoes is also interesting in view of the fact that cases of Mediterranean Fever occur amongst the military and naval garrison during the winter months. Looking at the question from this point of view the *Culex pipiens* would appear to be likely to play an important part in conveying infection.

PART II.—EXAMINATION OF MOSQUITOES TO DETERMINE WHETHER ANY SPECIES ACT AS CARRIERS OF THE *Micrococcus melitensis*.

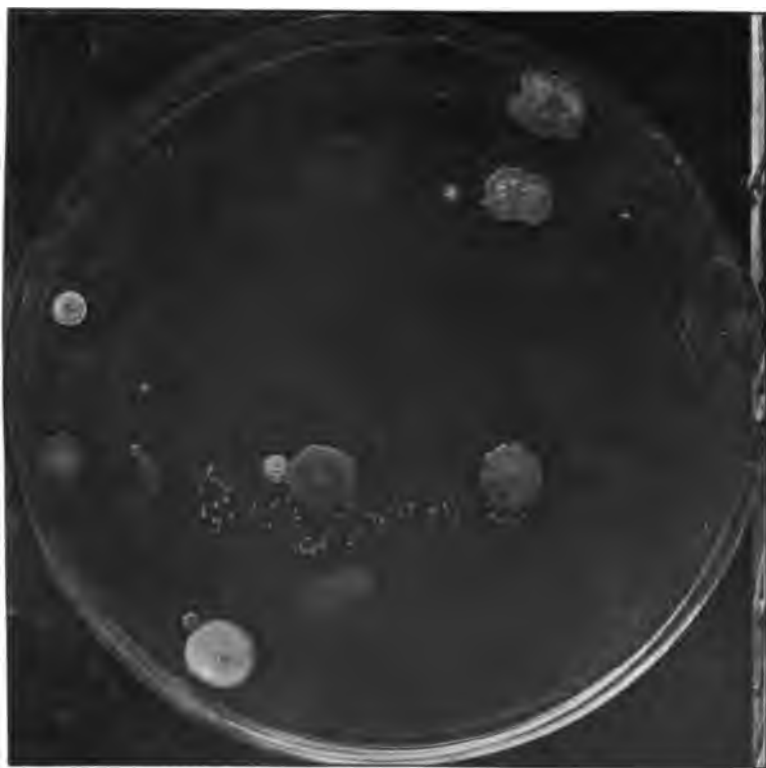
The following procedure was adopted in the examination of mosquitoes. Specimens full of blood were caught in boxes and stupified with chloroform. Each mosquito was then placed on a sterile glass slide, and needles being placed on the thorax and penultimate segment of the abdomen, gentle traction was exerted. With a little practice it was found possible to completely separate the parts, and leave the coagulated blood on the slide practically free from all contaminating fluids contained in the body of the mosquito. A few drops of sterile water were now added to the blood, and the whole was thoroughly mixed. The fluid was then drawn up in a sterile pipette, and transferred to litmus-nutrose-glucose-agar plates, and carefully spread over the surface of the medium. The plates were then incubated at 37° C., and examined in the usual way at the end of four days.

The first recovery was made on September 4 by one of us (H) from a mosquito (*Culex pipiens*) caught full of blood in the Lazaretto. One plate made in the manner described above contained 34 colonies of the *Micrococcus melitensis*. The micro-organism recovered was subjected to the most rigorous tests, and its pathogenicity was proved by injecting the growth on an agar slope obtained from one of the colonies on the plate (see Monkey C).

The second recovery was made on September 15 (by K) from a *Stegomyia fasciata* caught full of blood in the Mediterranean Fever wards of the Military Hospital, Valletta. One plate contained 24 colonies of the *Micrococcus melitensis*. The pathogenicity of this culture was also tested (see Monkey 101).

The third recovery was made (by H) on September 23 from a *Culex pipiens* caught in the Mediterranean Fever ward at the Naval Hospital, Bigli (Plate 2). The plate contained 100 colonies of the *Micrococcus melitensis*.

The fourth recovery was made (by H) on September 24 from a *Culex pipiens* caught in the Civil Hospital, Florian. The plate in this case only contained four colonies of the *Micrococcus melitensis*.



Blood from the stomach of a *Culex pipiens* caught in the Naval Hospital, Bighi, Malta. Plated on glucose-nutrose-litmus-agar. Incubated for 4 days at 37° C.

From photo. by Staff-Sergeant Rossiter, R.A.M.C.

It was noticed that, though several plates were inoculated with the blood from the mosquito, all the colonies on each occasion appeared in one plate.

Bearing in mind the work done by Shaw, Zammit, and Gilmour on the blood of patients suffering from Mediterranean Fever, the number of colonies which appeared to be present in the small quantity of blood contained in the mosquitoes is very remarkable, and suggests that either the *Micrococcus melitensis* undergoes multiplication in the mosquito, or else the micrococci are phagocyted in corpuscles which are broken up by the manipulations on the glass slide.

The following tables show the number and species of the mosquitoes which have been dissected up to the present time:—

Table A (Mosquitoes dissected by H.).

	<i>Culex pipiens.</i>	<i>Culex fatigans.</i>	<i>Stegomyia fasciata.</i>	<i>Acartomyia Zammitii.</i>	Total.
Military Hospital, Valletta	6	0	3	11	20
Civil Hospital, Floriana	63	0	24	0	87
Naval Hospital, Bighi	13	0	7	5	25
Military Hospital, Citta Vecchia	93	0	2	0	95
Military Hospital, Imtarfa	6	0	0	0	6
Barrack Room, Citta Vecchia	8	0	0	0	8
Military Hospital, Forrest	4	0	2	0	6
Military Hospital, Cottonera	13	0	0	0	13
Barracks, St. Andrews	4	0	0	0	4
Barracks St. Elmo.....	0	0	3	0	3
Lazaretto, Manoel	3	0	1	0	4
Barracks, Floriana	4	0	0	0	4
Total	217	0	42	16	275

Three recoveries of *Micrococcus melitensis* were made out of 275 mosquitoes dissected. It should be noted, however, that 95 mosquitoes were collected at Citta Vecchia, where the cases of Mediterranean Fever are mostly chronic, Citta Vecchia being a kind of sanatorium for the acute cases treated in Valletta and Cottonera. There were also few acute cases in the Civil Hospital, Floriana, at the time when the 87 mosquitoes were collected there.

Table B (Mosquitoes dissected by K. up to end of October, 1905).

Hospital.	<i>Culex pipiens.</i>	<i>Culex fatigans.</i>	<i>Stegomyia.</i>	<i>Acartomyia.</i>	Total.
Military Hospital, Valletta	109	3	45	32	189
Cottonera	5	1	—	—	6
Citta Vecchia.....	159	—	1	—	160
Imtarfa	20	—	—	—	20
Civil Hospital, Valletta	40	—	14	—	54
Naval Hospital, Bighi	2	—	—	—	2
Total	335	4	60	32	431

The mosquitoes in Table B were all captured in wards containing Mediterranean Fever patients.

Out of the total of 431 from these infected places, only one was found to contain *Micrococcus melitensis*. This was a *Stegomyia*, caught in 20A Ward, Valletta Military Hospital, on September 15. Twenty-four colonies of the microbe were obtained from the stomach.

Table C.—Mosquitoes caught in places where there were presumably no Mediterranean Fever Patients (dissected by K. up to the end of October).

Place.	<i>Culex pipiens.</i>	<i>Culex fatigans.</i>	<i>Culex Spathipalpis.</i>	<i>Stegomyia.</i>	<i>Acartomyia.</i>	Total.
Military Hospital, Valletta	58	1	—	9	4	72
Military Hospital, Cottonera	8	1	—	1	—	10
Military Hospital, Imtarfa	6	—	1	—	—	7
Military Hospital, Citta Vecchia	44	—	—	—	—	44
Military Hospital, Forrester	4	1	—	4	3	12
Floriana Barracks	9	—	—	—	—	9
Lower St. Elmo Barracks	6	—	—	3	13	22
Fort Ricasoli	7	1	—	2	1	11
Gozo	—	—	—	—	1	1
Valletta	1	—	—	1	—	2
Total	143	4	1	20	22	190

No *Micrococcus melitensis* was recovered from any of these.

The *Micrococcus melitensis* was only recovered from four out of a total of 896 mosquitoes dissected. It must, however, be noted that some 255 mosquitoes were obtained from Citta Vecchia, where the cases of Mediterranean Fever are mostly chronic, and about 200 other mosquitoes were caught in places where there were no known cases of Mediterranean Fever. Deducting these numbers from the total, the result would be four infected mosquitoes out of about 450 mosquitoes collected in presumably infected places.

This result was not unexpected; considering the small numbers of the specific micrococci which are found in the peripheral blood of Mediterranean Fever patients, mosquitoes could not possibly be infected in great numbers, or Mediterranean Fever would be much more prevalent than it is at present.

Experiments to Test the Virulence of the Micrococcus melitensis isolated from Culex pipiens and Stegomyia fasciata.

Culex pipiens. Monkey C.

The monkey used in this experiment was brought from Calcutta and kept under observation for nearly two months before the experiment was commenced. Its blood was tested on many occasions, but no signs of a reaction with the *Micrococcus melitensis* were observed. On September 4 one of the colonies on the nutrose-agar plate, made with blood from the *Culex pipiens* caught in the Lazaretto, was planted on an agar slope, and on September 19 the growth resulting, emulsified in salt solution, was injected subcutaneously into Monkey C. A wave of fever followed, and on September 29 the blood reacted in a dilution of 1/10. On October 11 the blood serum, diluted 1/50, was found to cause immediate agglutination of the *Micrococcus melitensis*. On October 14 the monkey was killed. Cultures were made from the spleen, kidneys, liver, bile, mesenteric, femoral, and axillary glands, and hearts' blood. The micro-organism was recovered from the spleen and heart's blood; it was also found in considerable quantity in all the plates from the glands.

This monkey was also used for experiments on the conveyance of infection by mosquitoes.

Remarks.—The wave of fever, blood reaction, and recovery of *Micrococcus melitensis* from the organs show that the culture isolated from *Culex pipiens* was undoubtedly virulent.

Stegomyia fasciata. Monkey No. 101.

Monkey No. 101 had been under observation for several weeks, and its blood repeatedly tested, with negative results, before the experiment was commenced.

The *Micrococcus melitensis* was isolated from the mosquito on September 15 and sub-cultured on September 19. On September 24 an emulsion of the growth on an agar slope was injected subcutaneously into Monkey No. 101. A well-marked wave of fever resulted. On the sixth day after the injection the blood serum gave a complete reaction in a dilution of 1/10, and the temperature rose to 106°·8. On October 15 the temperature fell to the "normal level," but a few days later an irregular secondary wave of fever set in and the agglutination reaction increased. On October 22 the working dilution of serum was 1/50, and on the 29th 1/300. On November 12 the monkey was killed with chloroform. The blood serum, tested just before death, was found, when diluted 1/1000, to cause immediate agglutination of the *Micrococcus melitensis*.

The microbe was recovered in great profusion from the spleen and lymphatic glands, in less quantity from the liver, and very sparsely from the kidney. Each of six broth tubes, inoculated with 0·5 c.c. of blood from the heart, was found to contain the micrococcus.

This monkey was also used for experiments on the conveyance of infection by mosquitoes.

Remarks.—The marked wave of fever, strong blood reaction, and the presence of the *Micrococcus melitensis* in the blood and all the viscera show that the culture isolated from the *Stegomyia fasciata* was very virulent.

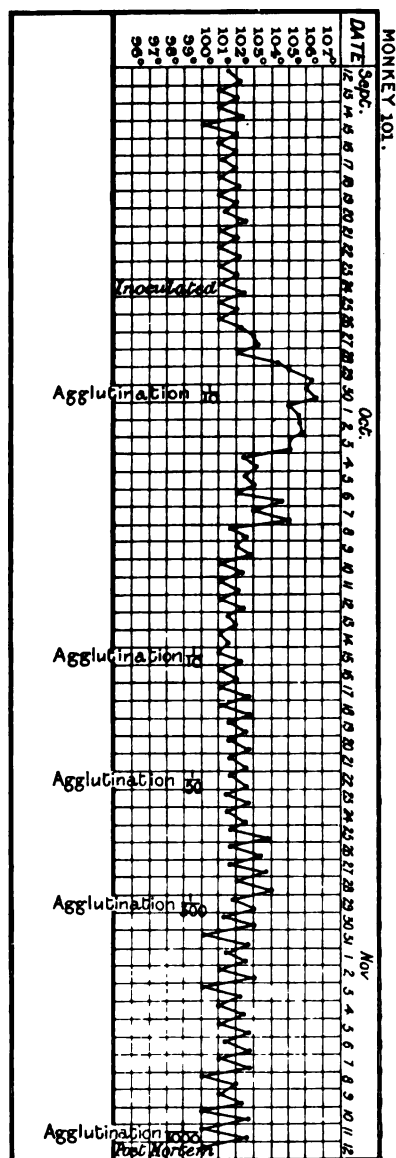
The chart (p. 77) shows the wave of fever during the experiment.

PART III.—TO DETERMINE WHETHER *Culex pipiens* AND *Stegomyia fasciata* CAN CONVEY THE INFECTION OF MEDITERRANEAN FEVER FROM MONKEY TO MAN, MAN TO MONKEY, AND MONKEY TO MONKEY.

Conveyance of Infection from Monkey to Man.

This experiment was not designed, but occurred in the following manner. On October 1 Monkey No. 6, infected by feeding, was being examined (by H.) in the verandah of the Lazaretto. Carlo Mifsud, the attendant in the Lazaretto, suddenly pointed to a mosquito, full of blood, resting on the table, and said, "that mosquito has just bitten me and I could not brush it away without letting go the Monkey." The mosquito was at once captured and dissected, with the results already detailed under the first recovery of the *Micrococcus melitensis* from *Culex pipiens*. On the day the mosquito was captured Carlo's blood was carefully examined, but no reaction with the *Micrococcus melitensis* was obtained. His temperature was also taken and found to be perfectly normal. The boy declared that he was perfectly well, and was much amused by the examination to which he was subjected. On October 11

he complained of feeling ill and returned to his home in Birchircara. On October 18 he was found to be suffering from fever and his blood



serum caused immediate agglutination of the *Micrococcus melitensis*. The boy still has fever (November 20), temperature ranging between 102° and 103°, and is undoubtedly suffering from a typical attack of

Mediterranean Fever. He stated that he had constantly drunk raw goat's milk obtained from F—— of Balzan; but for some 12 weeks before his illness he was employed from 7 A.M. to about 8 P.M. in the Lazaretto, and whilst working there he obtained milk from healthy goats which were being kept under observation for future experiments. He also stated that there were no cases of fever in his house at Birchircara. The infection of the mosquito might easily have been acquired from the infected monkeys in the Lazaretto. At the time there were several monkeys which had been infected by feeding and by subcutaneous injection, and whose blood yielded the *Micrococcus melitensis* on culture. As soon as monkeys became infected it was the custom to bring them daily out of the dark mosquito-proof rooms into the verandah for better observation. It is not difficult to imagine that the mosquito which bit "Carlo Mifsud" had previously fed on these monkeys and so had taken the *Micrococcus melitensis* into its stomach.

Conveyance of Infection from Cases of Mediterranean Fever occurring in Man to Healthy Monkeys.

Experiment I.—Conveyance of Infection by Culex pipiens. On August 21 a batch of mosquitoes bred out from larvæ were fed on a patient in the Valletta Hospital. There were about 50 of them in the cage and many were found full of blood. On the evening of August 23 they were fed on Monkey No. 22. On the same day another batch of mosquitoes were fed on a patient in the Valletta Hospital; on August 24 and 25 this batch was fed on Monkey No. 22. The feedings were done in the evening, but it was noticed that very few of the mosquitoes attacked the monkey. It was therefore decided to place the healthy monkey in a mosquito-proof cage and let the mosquitoes loose inside the cage. Accordingly, on August 31 this was done and another batch of mosquitoes fed on a patient was also let loose at the same time. On September 4 another batch was set free in the cage. On September 19 still another batch of mosquitoes was let loose. A jar full of water was placed on a bracket inside the mosquito cage, and many larvæ were eventually found in the water. The blood of Monkey No. 22 was carefully examined once a week for a reaction until October 25, when a severe attack of dysentery set in. On November 17 the monkey, being *in extremis*, was killed with chloroform. At the *post-mortem* examination miliary tubercles were found in the organs, and the liver was "waxy." Cultures were made in the usual manner, but the *Micrococcus melitensis* was not isolated.

Remarks.—In this experiment over 250 mosquitoes were employed, but not more than half of them absorbed blood from the Mediterranean Fever patients. Careful examination of the mosquitoes in the cage occupied by the monkey was made from day to day, but none of them were noticed full of blood.

Experiment II.—This was conducted on the same lines as No. I, with the exception that the monkey was placed in a small mosquito-proof box instead of a large cage, the idea being to bring the mosquitoes in more intimate contact with the monkey. The box had a double bottom, the upper being made of narrow bars and the lower of solid wood. Flat dishes containing water were placed in the space below the narrow bars. At intervals of 48 hours mosquitoes were placed in the cage. About 300 mosquitoes, which had fed on patients, were used, but of these only 80 containing blood were counted. Monkey No. 18 was used in this experiment and its blood was tested weekly for a reaction with the *Micrococcus melitensis*. It became seriously ill in October and was chloroformed on November 17. At the *post-mortem* examination a condition of things very similar to Monkey No. 22 was found. Cultures were made in the usual manner, but no signs of *Micrococcus melitensis* appeared.

Conveyance of Infection by Culex pipiens from Infected to Healthy Monkey.

A small box covered with mosquito netting was divided into two compartments by wire-netting fastened below to a board let into a groove in the floor, a false bottom made of wire was put in each compartment. These precautions were taken in order to prevent the passage of urine from one side of the box to the other. Monkey C, suffering from a wave of fever, having been placed in one compartment and Monkey No. 3, quite healthy, in the other about 400 mosquitoes were let loose in the compartment occupied by Monkey C. Next day 200 more mosquitoes were introduced. Unfortunately Monkey No. 3 died suddenly four days after the experiment was commenced. Another healthy monkey was then placed in the box and every day for a week batches of about 200 mosquitoes were let loose in the cage. The healthy monkey never showed any signs of infection.

Conveyance of Infection by Stegomyia fasciata.

Experiment I (from man to monkey).—Monkey No. 16 was placed in a large mosquito-proof box, and 200 mosquitoes, which had fed on patients in the Cottonera Hospital, were placed in the cage. The mosquitoes remained alive for a week. The blood of the monkey was repeatedly examined, but no signs of a blood reaction were observed, though the monkey was kept under observation for two months.

Experiment II (from man to monkey).—A cage containing over 100 mosquitoes was placed on a patient in the Cottonera Hospital on October 2. On October 4, 5, and 6 the cage was placed on Monkey No. 17. On each occasion some of the mosquitoes were noted to feed freely. On October 6 a cage full of mosquitoes, fed 48 hours previously on a patient in the Valletta Hospital, was placed on the monkey. On

October 7, 8, 9, and 10 the cage was again applied to the monkey. The blood of Monkey No. 17 has been subjected to repeated examination, but no signs of a blood reaction have been observed up to the present time.

Experiment III (from monkey to monkey).—On September 30 a cage full of mosquitoes, bred from larvæ, was placed on Monkey No. 101, then at the top of a wave of fever. Next day the cage was again placed on Monkey No. 101, so as to ensure as far as possible that all the mosquitoes should have an opportunity of taking up blood. On October 3 and 4 the mosquitoes were fed on Monkey No. 14. On October 5 and 6 the mosquitoes were again placed on Monkey No. 101, but on both days many dead mosquitoes were found in the cage. On October 7 the mosquitoes still alive were fed on Monkey No. 14. On October 9 all the mosquitoes were found dead. Monkey No. 14 has been kept under observation up to the present time, but the serum has never reacted.

Remarks.—The results of the experiments were disappointing, but not unexpected. The dissections of mosquitoes obtained from the wards containing Mediterranean fever patients, showed that under the most favourable conditions not more than 1 per cent. would carry the *Micrococcus melitensis*. In the experiments on the conveyance of infection from man to monkey, endeavours were made to feed as many mosquitoes as possible, but it was practically impossible to feed more than 300 mosquitoes at one time on the patients in the wards, and of these probably not more than one-half would take up blood at the first feeding; so that even with this large number of mosquitoes there was only a probability that one would carry the infecting microbe.

In the conveyance of infection from monkey to monkey, the feelings and interests of the patients did not militate against the use of any desired number of mosquitoes, but the monkey experiments presented their own peculiar difficulties. The attempts to isolate the *Micrococcus melitensis* from the blood of infected monkeys clearly showed that the specific microbe was present in the blood to a small extent, and that it appeared at very uncertain periods after infection, as judged by the serum reaction, had taken place. The *Micrococcus melitensis* also did not seem to persist in the blood for any long period after its recovery. From the fifth to the tenth day after the appearance of the agglutination was found to be the best time for its recovery from the blood. Though often the attempts made during life to isolate the micrococcus proved failures, yet the specific microbe was occasionally found in the blood obtained at the *post-mortem* examination. Again, monkeys displayed an enormous difference in their powers of resistance to infection; for instance, Monkey No. 110, intended for mosquito experiments, could not be infected, though he received subcutaneously at various times the growth of *Micrococcus melitensis* on six agar slopes. Mosquitoes also did

display the same predilection for monkeys as they did for man. It was often noticed that a cage full of *Culex pipiens* might be left for two hours in contact with a monkey, and not a single mosquito would bite. If, however, the same cage were transferred at once to the skin of man, the mosquitoes would commence to feed.

Conclusions drawn as to the Mode of Entrance of the Micrococcus melitensis into the Human Body, based on the Work done up to End of November, 1905. (W. H. H.)

(1) There is no evidence that Mediterranean Fever can be contracted by contact with cutaneous surfaces uncontaminated by urine.

(2) Infection can be acquired by the absorption of urine secreted by cases of Mediterranean Fever, and this is probably one way in which workers in hospital become infected.

(3) There is evidence to show that monkeys can be infected by dry dust artificially contaminated with cultures of *Micrococcus melitensis* isolated from the spleen of cases of Mediterranean Fever. The path of absorption may be through the nares, throat, respiratory passages, and alimentary canal. Dry dust contaminated with the urine of cases of Mediterranean Fever has given rise to infection in goats, but not in monkeys, up to the present time. The experience gained during the work performed in Malta during 1904-5 has convinced me that men are more susceptible to infection than monkeys and goats. Shaw's work on ambulatory cases of Mediterranean Fever amongst the Maltese has also shown that opportunities for the creation of infected dust are plentiful in Malta. Infected dry dust as a cause of Mediterranean Fever cannot therefore be discarded. When infection is acquired in this manner the incubation period is probably at least a month.

(4) Mediterranean Fever can be acquired by the absorption of infected goats' milk from the alimentary canal. The incubation period in this case is also probably long, and may even extend to two months.

This mode of infection probably plays a great part in the causation of Mediterranean Fever amongst the Maltese, who drink raw milk drawn at the doors of their houses.

(5) *Culex pipiens* and *Stegomyia fasciata* act as carriers of the *Micrococcus melitensis*, and the case of Carlo Mifsud renders it extremely probable that human beings are infected by the bites of infected mosquitoes.

(6) I believe that infected goats and infected mosquitoes play a greater part in the causation of Mediterranean Fever than the absorption of infected dust.

Preventive Measures.

If the conclusions drawn as to the mode of entrance of the *Micrococcus melitensis* into the human body be accepted, preventive measures should obviously be based on the following lines:—

(1) *Destruction of Infected Goats in Malta.*—The best indication of infection appears to be the milk agglutination test suggested by Zammit; unfortunately the test requires to be performed by a worker of considerable experience, and, judging by my own work, I think the hanging drop is preferable to the capillary tube employed by Zammit. The serum test is easier to perform, and as the experimental work has shown that goats may have a marked blood reaction, and that the *Micrococcus melitensis* may be present in the blood without the specific microbe necessarily appearing in the milk, all goats showing a decided serum reaction (dilution 1/20) should be destroyed. Examination of the milk alone cannot be taken as a basis of action in relation to goats, as we know that the excretion of the *Micrococcus melitensis* in the milk may be intermittent, and goats may be infected for some two to three months before the *Micrococcus melitensis* appears in the milk.

As goats may become infected by eating rubbish polluted with urine in the streets of Malta, and they themselves, when infected, excrete the *Micrococcus melitensis* in the urine, so, adding to the contamination of the public thoroughfares, it is plain that the perambulation of goats through the streets of Malta should be forbidden. The goats should either be milked in their pens, and the milk transmitted to the chief towns in sealed cans, or the goats should be assembled in some central dépôt outside the towns, and the yard of the dépôt should have a cemented surface, which can be thoroughly cleansed after the milking operations are over. As there is a strong probability that infection is also carried from infected human beings to goats by mosquitoes, the keeping of goats in houses, and in small yards attached to houses, should be forbidden. Goats should be kept in pens as far away from human habitations as circumstances will allow.

(2) *Destruction of the Larvæ of Mosquitoes.*—This is a large order in a place like Malta; but it must be attempted if the disease is to be stamped out. By means of pamphlets, householders should be instructed to apply oil to the surface of all stagnant water on their premises. About 15 c.c. of oil are sufficient for a square metre of surface; the application should be repeated every 15 days in the hot weather. The oiling of stagnant water in the houses of the poor should be performed by the sanitary authority.

(3) Promiscuous micturition about the streets should be forbidden, and a heavy penalty inflicted on any offender. By means of leaflets, the people should be educated to understand the importance of preserving some degree of sanitation in their dwellings, especially in relation to cleansing and flushing w.c.'s. Second class water for flushing purposes should not be made a means of revenue, but should be supplied at cost price.

VIII.—EXPERIMENTS ON MOSQUITOES AND FLIES.

By Captain J. CRAWFORD KENNEDY, R.A.M.C.

(Received December 16, 1905.)

I.—EXPERIMENTS WITH THE OBJECT OF INFECTING MOSQUITOES ARTIFICIALLY AND OF ATTEMPTING THEREAFTER TO INFECT A MONKEY.

Mosquitoes which had fed on uninfected people were collected full of blood and kept alive for several days by feeding them on a thick emulsion of *Micrococcus melitensis*, thereafter they were fed at regular intervals on Monkey No. 19. This animal had formerly been used for the *Stomoxys* experiment (see p. 66), but the last exposure to infection was on September 17, 25 days before this experiment was started, and no reaction had been detected in its blood.

Between the period from October 12 to November 3 about 40 mosquitoes, which had been treated as just mentioned, fed on this monkey. On October 5 the animal was very ill with dysentery, but no trace of reaction was found in its blood. On the 8th it died, and though a careful *post-mortem* examination was made of all its organs, no *Micrococcus melitensis* was recovered.

Dissections of the infected mosquitoes were made occasionally, and *Micrococcus melitensis* was recovered from the stomach once and from the thorax once; unfortunately in the latter instance the œsophagus had not been excluded from the muscular tissue.

II.—OBSERVATIONS ON *Stomoxys calcitrans*.

During the summer, when visiting the various herds of goats for the examination of blood and milk, I found that the sheds where the goats were housed were infested with a fly that resembled the common house fly, but had a piercing proboscis. I took this to be a *Stomoxys*, and this observation was kindly confirmed by Mr. Austen, of the British Museum, to whom I sent specimens, and who informed me that it was the *Stomoxys calcitrans*, but slightly smaller than the British variety. This fly is to be found all over Malta, and even in houses in town. It attacks viciously both animals and human beings, and its bite is very irritating; it sucks the blood of its victims, and is capable of holding a considerable quantity. Thinking that this fly might have some share in conveying the disease from goat to goat, I had supplies collected from a goat shed where a large number of the goats were infected, and carried out the following experiments.

Experiment 1.—Monkey No. 129, uninfected; blood gives no reaction. From August 15 to 20, flies straight from the goat shed were fed on this monkey; 25 flies bit well. From August 21 to 24 the same flies were fed on Monkey No. 107 (an infected monkey) before being again fed on Monkey No. 129. Monkey No. 129 was bitten by seven of these.

On August 25, Monkey No. 129 unfortunately died from peritonitis. No trace of *Micrococcus melitensis* was found in any of its organs, nor did its blood react.

Experiment 2.—Monkey No. 19, uninfected. Blood does not react. On August 25, 20 freshly caught flies were fed on this monkey. During the period between August 28 and September 15, 60 flies, which had been regularly fed on infected monkeys, were at intervals fed on this monkey. The animal has never shown any sign of reacting to *Micrococcus melitensis*.

Experiment 3.—Monkey No. 21, uninfected. Blood does not react. During the period between August 30 and September 7, 50 flies, which had been fed on infected monkeys, were thereafter fed on this monkey. The animal never showed signs of an agglutinative reaction, and died on September 22 (15 days after the last feeding) from peritonitis and tubercle of the lung. No *Micrococcus melitensis* was recovered from any of its organs at the *post-mortem* examination.

As the cooler weather approached it became increasingly difficult to obtain these flies in sufficient quantities for experimental work, and therefore further work is postponed.

Dissections.—Several of the flies were dissected out, and the various organs planted out on Petrie dishes, but no *Micrococcus melitensis* was recovered from any of these.

IX.—EXAMINATION OF ANIMALS IN CONNECTION WITH MEDITERRANEAN FEVER.

By Captain J. CRAWFORD KENNEDY, R.A.M.C.

(Received December 16, 1905.)

I.—THE EXAMINATION OF DOGS.

A series of 114 stray dogs, which had been seized by the police on the streets or in the suburbs of Valletta, Floriana, and Sliema, were examined. The animals were kept for 24 hours, in case they should be claimed by possible owners, and were then destroyed. Preparatory to their destruction, I examined their blood for an agglutinative reaction to the *Micrococcus melitensis*, and if a positive reaction were obtained, I made a *post-mortem* examination.

The agglutinative reaction was tried in dilutions of 1/10 and 1/30, with a time limit of half an hour. No sample was found to agglutinate beyond 1/30, unless it had stood for two hours.

The result was a positive reaction in 15 cases out of the total of 114, being a percentage of 13.15.

The following table gives at a glance the degree of reaction obtained. Each dog is described by a number which is placed in the column corresponding to the degree of the serum reaction.

	Reaction incomplete, 1/10.	Reaction complete, 1/10.	Reaction complete, 1/30.
Dogs by numbers	16 23 47 (no <i>post-mortem</i>) 76 83 111	3 11 73 90 101 103	35 44 107
Total	6	6	3 = 15

The degree of reaction is in no instance high, and but for the result of the *post-mortem* examination might very well be ignored.

Of the 15 dogs noted in the table, 14 were examined *post-mortem*. The routine adopted was to make cultivations on Petrie dishes from the following organs :—the spleen, the liver, the mesenteric, femoral, and axillary glands, and the urine ; broth cultures were also made from the heart's blood.

The result was that the *Micrococcus melitensis* was recovered in only one instance ; this was from No. 44, whose blood reacted up to 1/30

dilution, but did not go further. No. 44 was a dark brown short-haired bitch of Maltese breed, well advanced in pregnancy. Cultures on plates were made from the spleen, the liver, the mesenteric and femoral glands, and the urine. *Micrococcus melitensis* was recovered only from two plates made from the mesenteric glands. All the other organs were sterile and contained no micrococcus. The spleen was most carefully examined, practically the whole of the organ was cut into thin slices and smeared on six large Petrie dishes. The two plates made from the mesenteric glands contained respectively one and 34 colonies of *Micrococcus melitensis*.*

Summary.—None of the 114 dogs had the disease in an acute form.

One dog contained *Micrococcus melitensis* in its mesenteric glands.

At least nine (omitting the six with an incomplete reaction) showed unmistakable signs of infection—a percentage of 8.

Conclusions.—Sufficient proof is here presented that dogs become infected by the microbe of Mediterranean Fever. Although I have not happened to come across one with the disease in an acute form, and have not been able so far to demonstrate the presence of the *Micrococcus melitensis* in the excretions, still there is no reason to suppose that infected dogs do not excrete the microbe in their urine as do other infected animals. The importance of this source as another cause of infection will be readily recognised, and in this connection it is interesting to note that 3410 stray dogs were seized and destroyed by the police during last year in Malta alone.†

I am informed by Mr. Curmi, the Superintendent of Police, that the total number of dogs in Malta and Gozo is at least 40,000.

II.—PRELIMINARY NOTE ON MULES AND MALTESE STABLE EMPLOYÉS.

With the kind permission of Colonel Winter, Director of Supplies and Transport, I was enabled to examine the blood of 87 mules belonging to Government and used for transport purposes. The animals are not Maltese bred and have been in Malta for periods varying from 6 months to 10 years. They are groomed and driven by Maltese carters, and all the stable-hands are Maltese.

Those which I examined were quartered in three different stables:—

St. James's Ditch, Valletta.

San Marco, Valletta.

St. Paul's, Cottonera.

* Though it may be merely a coincidence that *Micrococcus melitensis* was recovered only from the mesenteric glands, it suggests that the infection had entered by way of the alimentary tract.

† Police Report, Malta, 1904-05.

St. James's Ditch Stables are the more modern and more sanitary buildings, well ventilated and airy, and are situated just outside the walls of Valletta.

San Marco Stables, standing in a low and thickly-populated part of the town, are poorly ventilated, shut in on all sides, and damp.

St. Paul's Stables are situated in the high ramparts of St. John's Bastion, in Cottonera lines. They overlook a slope thick with prickly pear, there is also a deep well just outside. The place is said to be infested with mosquitoes in the summer time.

I am informed that with the exception of some influenza and simple fever very little sickness occurs amongst the mules. In appearance the animals are sleek and in good condition.

On my first visit to the stables one of the mules which I examined (No. 42,290) gave a negative serum-reaction. On my second visit 12 days afterwards I was informed that this animal was on the sick list with slight fever, and had been off duty for a few days, but was returning to work next day. I therefore examined its blood again, and obtained a complete agglutination of *Micrococcus melitensis* in a dilution of 1/10, it did not, however, agglutinate in 1/20. This mule was undoubtedly suffering from a slight attack of Mediterranean Fever.

I have now (2 months later) examined its blood again, and find that it reacts completely in 1/10 and incompletely in 1/20 in half an hour. In the absence of any other means of proving the presence of the disease among these mules this was a very opportune case.

The following table gives the number of mules examined according to their service in Malta, and the stables in which they were quartered:—

Table A.

Service in Malta.	Quartered in stables at—			Total number examined.
	St. James's Ditch.	San Marco.	Cottonera.	
Under 1 year ...	6	—	—	6
1— 2 years.....	9	28	—	37
2— 3 "	2	—	1	3
3— 4 "	1	—	1	2
4— 5 "	1	—	1	2
5— 6 "	3	—	4	7
6— 7 "	9	1	6	16
7— 8 "	3	—	4	7
8— 9 "	1	—	3	4
9—10 "	2	—	1	3
Total	37	29	21	87

Each sample of blood was examined in dilutions of 1/10 to 1/50, with a time limit of half an hour. A reaction was obtained in 39 cases, or 44·8 per cent., and the following table (B) gives these worked out to their highest dilutions :—

Table B.

Dilutions ...	1/10.	1/20.		1/30.		1/40.	Total.
		In-complete.	Complete.	In-complete.	Complete.	In-complete.	
Number of mules which reacted	23	6	6	2	—	2	39

I was enabled, through the courtesy of Mr. Macfarlane, M.R.C.V.S., to make *post-mortem* examinations of three mules which had to be destroyed on account of age and unfitness. I had previously examined samples of their blood, and found that one (No. 43,013) reacted in dilution 1/20, another incompletely in 1/10, and the third not at all. Cultures were made from the spleen, the mesenteric and femoral glands, and also, in the case of No. 43,013, from the liver. In every case these organs contained no *Micrococcus melitensis*.

To assist in arriving at some conclusions from these observations I drew up the facts in tabular form as follows :—

Table C.—Number of Infected Mules by Service in Malta.

	Under 1 yr.	1-2 yrs.	2-3 yrs.	3-4 yrs.	4-5 yrs.	5-6 yrs.	6-7 yrs.	7-8 yrs.	8-9 yrs.	9-10 yrs.	Total.
Number of mules examined	6	37	3	2	2	7	16	7	4	3	87
Number which reacted	1	21	2	1	1	4	5	3	1	0	39

Table D.—Number of Infected Mules according to Stables.

	St. James's Ditch.	San Marco.	Cottonera.	Total.
Number of mules examined	37	29	21	87
Number which reacted	10	15	14	39
Percentage...	27	51·7	66·6	

Table E.—Number of Infected Mules of less than Two Years' Service, according to their Stables.

Mules under 2 years' service.	St. James's Ditch.	San Marco.	Cottonera.	Total.
Number examined	15	28	—	43
Number which reacted	7	15	—	22
Percentage...	46·6	53·5	—	51·1

A study of these tables suggests the following observations :—

(1) Mules of less than two years' service show a slightly larger proportion of infected cases than those of more than two years' service. This difference becomes much more marked as the reactions in the lower dilutions are eliminated, as shown in the next table (F). For the purposes of this comparison it is convenient that half the total number examined were under, and the other half over, two years' service.

Table F.

Number of mules.	Service in Malta.		Total.
	Under 2 years.	Over 2 years.	
Examined	43	44	87
Reacted in 1/10	22	17	39
„ 1/20	7	3	10
„ 1/30	4	—	4

It will be seen that none of more than two years' service reached the 1/30 dilution, while only three out of a total of 10 reached 1/20. This points to a more severe or more recent infection among the later arrivals in the island.

(2) The stables at San Marco and at Cottonera show a larger proportion of infected animals than those in the Ditch. This, in the case of San Marco, is, I think, more apparent than real, for this reason, that most of the mules of less than two years' service are quartered there. When the two stables (San Marco and the Ditch) are compared by mules of the same length of service, the difference almost disappears. At the Ditch there were 7 infected out of 15 examined = 46·6 per cent. At San Marco 15 out of 28 = 53·5 per cent.

At Cottonera all the mules are of more than two years' service, and comparing them with those of the same service at the Ditch, we find that the larger proportion of infected cases at Cottonera is undoubted. At the Ditch there were 3 infected out of 22 examined = 13·6 per cent. ; and at Cottonera 14 out of 21 = 66·6 per cent. There are therefore in proportion four times more infected mules in Cottonera than in the Ditch or in San Marco.

Summary.—Blood serum reaction to *Micrococcus melitensis* was obtained in 44·8 per cent. mules examined. In one case the appearance of the serum reaction was coincident with a slight attack of fever. The serum reaction obtained was in every case rather low, only two reaching 1/40.* The highest reactions were obtained in those of under two years' service in Malta. In proportion there were four times more infected mules in Cottonera than in the Valletta stables.

Conclusions.—Mules are exposed to and suffer in a mild way from infection by *Micrococcus melitensis*. I reserve other remarks until further investigation has been carried out.

The Maltese Stable Employés.

The examination of those employed in the stables was a natural sequel to the examination of the mules. They are all Maltese, who have been employed with the Army Service Corps for periods varying between 20 years and 20 months: a good many new hands were taken on 20 months ago, and of these I examined 33. The total number examined was 80. I have divided them into two classes according to their employment.

(1) *Carters.*—These men drive the mules and spend most of their day on the road. They also groom their mules and spend one night in 8 or 9 on stable guard, when they remain on duty in the stables all night. On other nights they sleep at their respective homes.

(2) *Stable-keepers or Labourers.*—These men are the sweepers and general labourers in the stables; they spend practically the whole day in and about the stables, and on one night in every three they sleep there.

The result of the examination of the blood of these men for a reaction to *Micrococcus melitensis* was as follows:—

1. *Carters.*—The total number examined was 73, and of these 30 had been only 20 months in the service. Four gave a reaction; two in 1/30 and two in 1/10 dilutions. Their particulars are as follows—

No. 80.—Reacted 1/30; 13 years employed; says that he had fever lasting 2 months 3 years ago, and that he is in good health now. Temperature when examined 99°·6.

* To compare low serum reaction in animals, *vide* Report on Malta Fever in Dogs, *ante* p. 84.

No. 121.—Reacts 1/30; 20 months employed; was sick with fever and pains in head 4 months ago.

No. 112.—Reacted 1/10; 20 months employed; had fever lasting 18 days 4 years ago; is in good health now.

No. 114.—Reacted 1/10; 14 months employed at Cottonera; says that he has never been sick except for pains in the head during the summer. Temperature normal.

Besides these cases, faint reactions were obtained in 3 others who had a history of fever many years ago, they were all old hands.

Nos. 80 and 121 are the only ones out of the above 4 that can definitely be said to have contracted fever whilst employed in the A.S.C. stables; and No. 121 is the only one out of 30 with 20 months' service who has taken the disease.

2. *Stable-keepers*.—The total number examined was 7, and of these 3 had 20 months' service. Two reacted. Their particulars are—

S. A——.—Reacted in 1/30; 20 months employed; fell sick at San Marco Stables in the beginning of the summer and was ill for 4 months.

S. V——.—Reacted in 1/10; 6 years employed; had fever some years ago.

Both these stablemen contracted the disease whilst employed in the stables, and S. A—— is one of three who have only 20 months' service.

Summary.—Of a total of 80 men examined, 4 (= 5 per cent.) showed signs of a more or less recent infection and had contracted the disease whilst employed in the stables. Two were stablemen and 2 were carters, being 28 per cent. of stablemen as compared with 2·5 per cent. of carters. Two out of 33, or 6 per cent., contracted the disease within 20 months of starting work at the stables, one of them was a stable-keeper, being 1 in 3 stablemen as compared with 1 in 30 carters.

Conclusions.—These observations are suggestive of lines of future work, especially with regard to biting insects.

X.—BACTERIOLOGICAL EXAMINATIONS OF CASES OF MEDITERRANEAN FEVER.

By Captain J. CRAWFORD KENNEDY, R.A.M.C.

(Received December 16, 1905.)

I.—EXAMINATION OF SALIVA.

The cases examined were four in number, and were selected on account of their severity.

CASE A.—A very severe case with persistent and irregular fever, no normal temperature for 90 days; extreme anæmia. Samples of saliva were taken from this case on the following days of disease, 36th, 37th, 38th, 39th, 41st, 42nd, 68th, 69th, 70th, 71st, 72nd, 73rd, 74th, 75th, 76th, 77th, 83rd and 84th.

CASE B.—A moderately severe case with four well-marked "waves" of fever. The third wave was the most severe. Samples of saliva were collected on the following days of the disease, during the comparative apyrexial period between the 3rd and 4th "waves," the 58th, 59th, 60th, 61st, 63rd and 64th days.

CASE C.—A severe case with marked anæmia. First "wave" severe, prolonged, lasting 30 days. Second "wave" very acute, lasting 21 days. Samples of saliva collected in the period between the 1st and 2nd "waves" on the following days of disease, the 42nd, 43rd, 44th, 45th, 47th and 48th.

CASE D.—First "wave" ran a regular course, with little constitutional disturbance, though temperature was high; it lasted 34 days. The samples of saliva were collected on the 38th and 39th days of disease during the apyrexial period before the second "wave."

Method of Collecting the Saliva.—It was attempted to collect the saliva as it escaped from the orifices of the ducts, but, on account of the inconvenience caused to the patients, this was not persisted in. The patient was therefore asked to spit into a sterile test-tube after his mouth had been well cleansed by means of a tooth brush and carbolic powder and well rinsed with a solution of boracic acid.

Method of Examination.—The saliva, being loaded with contaminating organisms, and, in especial, with acid producing *Streptococci*, requires to be greatly diluted before its culture on plates could be attempted with any chance of success. Two or three methods were tried, but the following was finally adopted:—

Four or five test-tubes, each containing 10 c.c. of distilled water, were sterilised and labelled respectively A, B, C, D, E. To A was added 1 c.c. of the saliva; to B 1 c.c. of the mixture in A; to C 1 c.c. of the mixture in B, and so on. The diluted saliva was then planted

out on Petrie dishes containing the nutrose litmus agar, 1 c.c. from each tube being distributed over four plates.

After incubation for four days the plates made from tubes A and B were, as a rule, found to be crowded with acid colonies, and quite useless. The higher dilutions gave very clean plates; 298 plates were thus examined, but *Micrococcus melitensis* was not recovered.

Result.—In 32 samples of saliva from Mediterranean Fever patients in varying stages of the disease, no *Micrococcus melitensis* was found.

II.—*Post-mortem* EXAMINATION OF FATAL CASES OF MEDITERRANEAN FEVER.

During the year 1905 a more or less systematic *post-mortem* examination of fatal cases has been carried out in order to obtain some idea of the distribution of the *Micrococcus melitensis* in the human body. A series of nine cases has been collected and to these I have added four others which occurred in the previous year. A number of these have been examined in conjunction with Major Horrocks. My observations and remarks are recorded concisely under the headings of the various organs, and the accompanying table will show at a glance the proportion of recoveries of *Micrococcus melitensis* from each organ.

Spleen and Liver.—These organs invariably contain *Micrococcus melitensis* in large quantities during the febrile stage.

Kidney.—The organism is not so abundant here and in one case was not obtained.

Bile.—Eight samples were cultured, and from two *Micrococcus melitensis* was recovered in pure culture, but in no great quantity.

Bile Duct and Bladder.—Scrapings were made from the walls in two instances. No recovery of the microbe was made.

Urine.—Last year, when the urine investigation was being carried on, three samples were taken from the bladder: one sample contained the microbe. This was the second occasion on which *Micrococcus melitensis* was found in the urine.

Intestines.—These were examined in four cases, and several hundred plates were made from scrapings from the walls or from the contents of the duodenum jejunum, ileum, and colon. The specific microbe was not detected, although it must be present, seeing that it occurs in the bile, but it evidently does not multiply in the intestines.

Bone marrow was examined in four cases. Recoveries of the microbe were made in two of these. In one of them the colonies of *Micrococcus* were very profuse.

Heart's Blood.—Cultures were made in four instances, two of which yielded the microbe. One of the unsuccessful cases was a chronic one of a month and a half's duration; in the other only a very small quantity of blood was used.

Pericardial Fluid.—In one case out of two *Micrococcus melitensis* was recovered ; this was one which had lasted for a month and a half.

Pleural Fluid.—One case was examined ; result, negative.

Cerebro-Spinal Fluid.—This was examined in two cases with marked meningeal symptoms. The fluid was much increased in quantity and had caused a good deal of flattening of the cerebral convolutions. Great difficulty was experienced in keeping it free from contaminations. No *Micrococcus melitensis* was recovered.

Lymphatic Glands.—A distinct advance has been made by the discovery that the lymphatic glands are great receptacles for the *Micrococcus*. In one of my later cases I was struck by the size and appearance of the mesenteric glands. They were similar to what are found in enteric fever—the size of a large marble, fluid in the centre and the capsule injected. On being cut, they were found to contain what was to all appearance pus. A loopful of this fluid was spread over several Petrie dishes containing the usual nutrient medium, and after incubating for four days a pure growth of *Micrococcus melitensis* resulted, so profuse as to cover every part of the plate that had been touched by the platinum wire. After this discovery the glands from other parts of the body were examined and also found to contain microbes in great quantity. In one case the glands from the axilla groin, mesentery, carotid and mediastinum were examined, but only from the mediastinal was the microbe obtained. In another case where the femoral, thoracic and mesenteric glands were examined, the microbe was present in the femoral and thoracic, but not in the mesenteric.

This observation has been of the greatest value in the *post-mortem* examinations of experimental animals. In many cases they take the disease in a mild form, and the specific microbe soon dies out of the spleen, remaining, however, for a longer time in the glands. Therefore the routine practice at *post-mortem* examinations is to examine the femoral, axillary and mesenteric glands, as well as the other organs. Had this not been done many *post-mortem* examinations must have proved negative, seeing that the glands were the only organs which contained *Micrococcus melitensis*.

The following idea is suggested by the foregoing observations. If the mode of infection happen to be through a mucous membrane, *i.e.*, the alimentary canal, the glands draining that area offer a good nidus for the multiplication of the *Micrococcus melitensis*, and in time form a good focus from which the whole system becomes infected. I think that many cases with long incubation periods might be explained in this way: For instance, a common type of the disease is manifested as follows:—

First, a slight attack of fever, labelled, for want of a better term, S.C. Fever, lasting two, three, or four days, with no agglutinative reaction in the blood. This probably coincides with the local invasion.

Second, a period of quiescence lasting three weeks to a month or longer, the period of incubation.

Third, sharp attack of high fever, with no appreciable enlargement of the spleen till the third or fourth day. This marks the systemic invasion and the full development of the disease.

Tonsils.—In view of the glandular character of these organs and a possible entrance for infection, four cases were examined, but no *Micrococcus melitensis* was isolated.

Salivary Glands.—Concurrently with the examination of the saliva, two cases were examined *post-mortem*, but without the recovery of the microbe.

Table and Summary.

Organ.	Number of times examined.	Number of times <i>Micrococcus melitensis</i> recovered.
Spleen	13	13 = 100 p. c.
Liver	3	3 = 100 "
Kidney	7	6 = 85 "
Urine	3	1
Lymphatic glands	5	5 = 100 "
Mediastinal	1	1
Thoracic	1	1
Mesenteric	5	3
Femoral	3	2
Axillary	1	0
Carotid	1	0
Heart's blood	4	2
Pericardial fluid	2	1
Bone marrow	4	2
Bile	8	2
Bile duct and bladder	2	0
Intestines	4	0
Duodenum	4	0
Jejunum	3	0
Ileum	3	0
Colon	1	0
Salivary glands	2	0
Tonsils	4	0
Pleural fluid	1	0
Cerebro-spinal fluid	2	0

XI.—AN EXAMINATION OF GOATS IN MALTA, WITH A VIEW TO ASCERTAIN TO WHAT EXTENT THEY ARE INFECTED WITH MEDITERRANEAN FEVER.

By Dr. T. ZAMMIT.

(Received December 18, 1905.)

I.—ATTEMPTS TO RECOVER *Micrococcus melitensis* FROM GOATS SLAUGHTERED AT THE CIVIL ABATTOIR.

As soon as goats were suspected to be liable to the infection of Mediterranean Fever, an examination of the animals slaughtered at the civil abattoir was undertaken. None of the animals had been previously selected or examined, and some of them turned out to be male Barbary goats which had been brought to Malta to be fattened and sold as mutton. When a goat was killed a capillary tube was filled with blood for the serum test, and the spleen was carefully removed and taken to the laboratory for examination; 46 goats were thus examined, and the microbe was recovered in one case only. The serum reaction showed, however, that more than one goat had at some time been suffering from Mediterranean Fever, for seven of the bloods gave a clear positive reaction with *Micrococcus melitensis* at a dilution of at least 1 in 80. All the goats appeared to be in good condition, and were passed for consumption by the veterinary surgeon of the abattoir.

II.—*Post-mortem* EXAMINATION OF GOATS BOUGHT IN JUNE, 1904.

The goats bought by us in June, 1904, and found to be suffering from Mediterranean Fever, were slaughtered in September, 1905. Of six animals, numbered one to six, No. 4 never gave a reaction for Mediterranean Fever, and was therefore used for other experiments. No. 3 always reacted strongly after it was sent to the Lazaretto. In July, 1905, it lost flesh, and on August 2, being in a dying condition, it was killed and carefully examined. A large number of broth and agar tubes were inoculated with material taken from all the organs, but the *Micrococcus melitensis* was not recovered. Nos. 1, 2, 5, 6 appeared quite healthy. They were slaughtered between September 25 and 29. The animals were in perfect condition, very fat, and with all the organs apparently healthy.

A great number of culture tubes were used for the examination, and, fortunately so, for if fewer had been used it is probable that the microbe would have escaped notice in some of the cases. The *Micrococcus melitensis* was recovered from all the goats except from No. 2. During life this micro-organism had only been obtained from the blood of Nos. 5 and 6, but on the other hand it had been recovered from the

urine and milk of all four goats. At the *post-mortem* examination the microbe appeared to be irregularly distributed in the body and in rather small numbers.

In No. 1 it was obtained from the spleen and the kidneys, but in small quantities. In No. 5 it was recovered from the kidneys only. In No. 6 it was only found in the glands.

Other goats (Nos. 15, 16, 17, 18), affected with Mediterranean Fever, were slaughtered in September. The *Micrococcus melitensis* was recovered from all except from No. 18. In No. 15 it was obtained abundantly from the kidneys. In Nos. 16 and 17 only a few colonies were procured from the lymphatic glands.

The serum reaction remained positive and constant in all the bloods, though in some of them it became very weak, not higher than 1 in 40.

The *Micrococcus melitensis* in infected goats tends to disappear from the system after a time, but the process is slow.

Our goats having been bought in June, 1904, already infected, we could not ascertain how long they might have been in that condition, but it is a fact that after 15 months the specific micro-organism was still living in their lymphatic glands.

III. RECOVERY OF *Micrococcus melitensis* FROM THE BLOOD OF GOATS.

In certain phases of the disease the specific microbe circulates freely in the blood, and it can then be easily recovered. This condition does not seem, however, to last long, since a blood which yields the microbe one day, will not show any after the lapse of a week. About 5 c.c. of blood were taken from the jugular vein of goats 32 times. The same animal was sometimes tried three or four times. The blood was distributed in 20 c.c. broth tubes and incubated. The *Micrococcus* was only recovered from four goats (Nos. 5, 6, 21, and 29). The animals had all contracted the disease before they were obtained, and as when brought to us they already displayed a strong serum reaction, no idea could be formed as to the stage of the disease at which they had then arrived. Some of the cases were undoubtedly of long standing.

IV. THE REACTION OF GOAT'S MILK TO THE *Micrococcus melitensis*.

On July 10, 1905, I observed that the agglutination test could be applied to the milk of goats affected by Mediterranean Fever as well as to the blood. At a point in our investigations it had become difficult to obtain samples of blood from goats, as the owners strongly objected to have their animals bled. The use of milk instead of blood for the specific reaction proved a great help and enabled us to examine a large number of goats.

The test can be applied on a slide in the ordinary way or in capillary pipettes as in the method of precipitation. In time, however, the precipitation method was adopted as being more conclusive and easier to work, especially when a great number of samples had to be dealt with.

The only precaution to be taken in applying the test is the addition of an antiseptic, strong enough to prevent the clotting of the milk, but without affecting in any way the agglutinins. For the examination of a large number of samples the following method was found to answer best:—

A strong emulsion of the *Micrococcus melitensis* is prepared in normal saline solution in a watch-glass. To this a small quantity of formaldehyde solution is added (one small loopful of a 1-per-cent. solution), the whole being drawn into a pipette. One drop of the emulsion is placed on a glass slide and a loopful of milk is mixed thoroughly into it. This mixture is then drawn up into a fine capillary pipette, left in an upright position for 12 hours, and the reaction noted at the end of that time. The reaction is often seen after a few minutes. The cream collects at the surface and does not interfere with the reaction.

Between July 10 and September 22, 1905, 710 samples of milk were examined and a positive reaction was obtained 133 times.

With a view to check the value of this method all the milks that showed a positive reaction were tested a second time. Further, where possible, blood was obtained from the animal for the serum test, and the suspected milk was plated out.

The blood test constantly confirmed the milk test, and when a strong reaction was obtained the specific microbe was always recovered from the milk. In conclusion, in my opinion, the milk test is a safe one and quite as reliable as the blood test. For sanitary purposes, more especially where a great number of goats have to be examined, the milk test is at once convenient and efficient.

V. THE MILK TEST AS APPLIED TO GOATS IN THE COUNTRY.

As soon as the susceptibility of the goat to Mediterranean Fever had been established it became obviously desirable to ascertain how far herds which supplied milk to the towns and villages were affected. In July, 1905, we heard from one of the district medical officers that cases of the fever were numerous in two villages, Lia and Balzan, whereas few or none were reported from the neighbouring village of Attard. These three villages form a group lying close together about the centre of the island.

It was decided, therefore, to examine the herds of this group of villages first and then work in other directions according to circumstances. Every village has a number of herds, but many goats are

distributed singly, every family, as a rule, keeping a goat for its own use. I have arranged the result of the examination in the following tabular form :—

Name of village.	Street.	Number of goats in herd.	Number of goats which reacted.	Remarks.
Lia	Molino Musta	15	None	
"	Forni, 12	7	2	
"	" 24	4	None	
"	Concezione	9	"	
"	Molino, 28	5	"	
"	Preziosi	13	"	
"	Concezione	11	"	
"	Reale, 9	1	"	
"	" 128	2	"	
"	Stretta Enea, 2	1	"	
"	" 3	6	"	
"	Concezione, 33	1	"	
"	" Vlo, 3°	2	1	<i>Micrococcus meli-</i>
"	" 28	4	None	<i>tensis</i> recovered.
"	Forni, 54	1	"	Case of Medi-
"	" 42	1	"	terranean Fever
"	S. Andrea, 15	1	"	on the premises.
"	No address	4	2	Goats were being
"	Reale, 82	12	7	taken to Mosta.
Balsan	Reale, Vlo, 2°	18	6	The <i>Micrococcus</i>
"	Provvidenza Vlo, 2°	20	2	recovered from 8.
"	" Vlo, 3°	7	None	2 cases of Medi-
"	" Vlo, 2°	17	3	terranean Fever
"	"	16	2	on the premises
"	3 Chiese, 25	18	2	during the last
"	" 9	1	None	twelvemonth.
"	" 33	1	1	
"	Itmeida, 16	1	1	Case of Mediter-
"	No address	3	2*	ranean Fever on
Attard	Via C. Cormi, 22	5	None	the premises.
"	" 2	2	"	*Goats were being
"	Via Notabile, 3	7	"	taken to Mosta.
"	Reale, 24	2	"	
"	Lunatic Asylum	31	"	
"	Molino Vlo, 1°	4	"	
"	" 14	5	"	
"	S. Domenico, 19	6	"	
Zeitun	Herba, 16	8	"	
"	Giardino Botanico, 10	10	"	
"	Vlo Privato, 3	6	"	
"	Piazza Maggiore, 44	12	"	
"	Madonna Pietà, 119	11	1	
"	Giardino B.	11	1	
"	Herba, 26	8	None	
"	Sciortino, 22	11	"	
"	S. Giovanni	5	1	
"	Sta Maria	5	None	
"	Marsascirocco, 11	5	1	
"	" 11 (A.C.)	11	None	
Zabbar	Xghira, 75	8	1	
"	Marsascala, 22	8	None	

Name of village.	Street.	Number of goats in herd.	Number of goats which reacted.	Remarks.
Zabbar.....	Marsascala, 6.....	18	5	<i>Micrococcus</i> recovered from 3.
"	Piazza S. Giacomo, 30 ...	11	1	
"	Sta Maria, 12.....	7	None	
"	Marsascala	13	3	
"	Vlo Hassajed	7	1	
"	Xghira, 72	5	3	
"	" 71	11	3	
"	S. Domenico, 1	4	1	
"	S. Giuseppe, 68	18	2	
"	Capuccini	14	1	
"	Marsascala	6	2	
"	"	14	9	
"	S. Domenico	10	7	
"	Tal Fgura	24	5	<i>Micrococcus</i> recovered from 2.
Hamrun ...	Dolori, 29	30	8	
"	Vlo Tal Fatati	18	11	
"	"	85	5	
Axisk	Via Gud'a	10	2	
Curmi	S. Pietro	8	1	
Taxbiex ...	Via Sliema	5	None	

The principal herds of Lia, Balzan, Attard, Zeitun, and Zabbar were visited, and about one-half of the goats of those villages were tested. The percentage of infected animals is therefore practically accurate. It stands as 12 per cent. for Lia, 19 per cent. for Balzan, 0 per cent. for Attard, 4 per cent. for Zeitun, and 25 per cent. for Zabbar.

As to Hamrun, only three herds were gone through, and there are hundreds of goats in that village. The percentage of infected goats at this place cannot be deduced from our work, but the three herds examined were badly infected.

XII. A CRITICAL EXAMINATION OF THE BLOOD OF PATIENTS IN HOSPITAL, TO DETERMINE IF OTHER THAN MEDITERRANEAN FEVER SERA WOULD AGGLUTINATE THE *MICROCOCCUS MELITENSIS*.

By Fleet-Surgeon P. W. BASSETT-SMITH, R.N.

(Received January 11, 1906.)

The importance of placing beyond doubt the specific character of the agglutination of the *Micrococcus melitensis* when brought in contact with the blood serum of patients, cannot be over estimated, either when the test is used for diagnosis, or for controlling experimental work. There have been cases, from time to time, which have led certain diagnosticians to underrate this modern method of diagnosis. These people would therefore naturally discredit all investigations based on this principle, pointing to cases in which contradictory results have been obtained from the same serum, and to statements that a positive reaction for Mediterranean Fever has been met with in other diseases.

Bearing these facts in mind, I have made a careful examination of 150 samples of blood, taken systematically in the wards of Haslar Hospital, for the purpose, if possible, of demonstrating whether or not the serum of patients suffering from a great variety of diseases other than Mediterranean Fever would give a reaction likely to render a mistake in diagnosis probable. It is unnecessary to describe fully the technique employed, this being so well known, excepting to say that—

1. The tubes containing the blood were centrifugalised, and the clear serum was alone used.

2. The emulsion was made from an agar culture 10 days old of a strain of *Micrococcus melitensis* obtained in November, 1905, from the peripheral blood of a patient now in the hospital, and was used living.

3. The serum dilution of 1 in 30 was made with normal saline solution, using accurately graduated pipettes.

4. The examination was made both microscopically, with a 4-hour limit, and by sedimentation tubes with a 24-hour limit.

5. Controls were made for each batch of tubes, with a serum that reacted perfectly in dilutions from 1 in 30 to 1 in 1000.

The whole examinations were made by myself, but the readings were confirmed by independent observers.

The results are tabulated as follows:—

Nature of disease.	Number of cases tested.	Microscopical.	Sedimentation.
Enteric fever	3	Negative	Negative.
Tubercle of lung	12	"	"
" testicle	1	"	"
" joint	1	"	"
Tubercular empyema	1	"	"
Pneumonia	5	"	"
Bronchitis	2	"	"
Bright's disease	3	"	"
Hydronephrosis	1	"	"
Rheumatism	7	"	"
M.C.O.	7	"	"
Tonsilitis	3	"	"
Dilated stomach, etc.	1	Positive	Positive.
Lead paralysis	1	Negative	Negative.
Appendicitis	7	{ 2 Positive 5 Negative	{ 2 Positive. 5 Negative.
Hemiplegia	1	Negative	Negative.
Epilepsy	3	"	"
G.P.I.	1	"	"
Alcoholism	1	"	"
Aneurism	1	"	"
Abcess, local	7	"	"
" liver	2	"	"
" peas	1	"	"
" mastoid	6	"	"
Cellulitis	4	"	"
Septic thrombosis	1	"	"
Otorrhœa	2	"	"
Iritis	3	"	"
Keratitis	2	"	"
Synovitis	1	"	"
Herniotomy	5	"	"
Hæmorrhoids	1	"	"
Varicose veins	1	"	"
Ulcers	2	"	"
Fractures	10	"	"
Wounds	4	"	"
Eczema	4	"	"
Gonorrhœa	5	"	"
Gon. rheumatism	2	"	"
Syphilis 1	16	"	"
" 2	7	"	"
Normal blood	2	{ 1 Positive 1 Negative	{ 1 Positive. 1 Negative.
Totals	150	{ 4 Positive 146 Negative	{ 4 Positive. 146 Negative.

It will be seen that the blood of 41 pathological conditions was tested, and that, in all but four cases there was no evidence of agglutination of the *Micrococcus melitensis*. Of these four positive reactions, two appendicitis cases had lately returned from Malta Hospital, and were running a regular undulant temperature, and had undoubted

Mediterranean Fever. The third case was a sick berth steward, who had Mediterranean Fever two years and 10 months ago. The fourth was a long time in Malta Hospital, where gastroduodenostomy was performed, and, though there is no definite temperature chart of Mediterranean Fever, I have no doubt that he was, like so many others, infected by the micro-organism there. His temperature is now irregular.

All these examinations, therefore, gave an *absolute negative* to other than Mediterranean Fever blood causing agglutination of the *Micrococcus melitensis* in a dilution of 1 in 30. The following points were also investigated with regard to this reaction:—

Will Lower Dilutions give Erroneous Results?—Ten of the already-used samples of blood were tested in dilutions of 1 in 5, 1 in 10, and 1 in 20. In one case only was there any reaction, an abscess of the knee, which agglutinated up 1 in 10.

Are the Agglutinating Properties Destroyed by Keeping the Blood?—Some serum from a tube of blood, which had been taken from a patient in November, 1901, was tested in the same manner.

	Dilution.			
	1/10.	1/20.	1/40.	1/200.
Microscopic	+	+	+	+
Sedimentation	+	+	+	+
Control, normal blood...	—	—	—	—

Result.—Four-year old blood serum agglutinated perfectly.

Are the Agglutinating Properties Destroyed by Heat?—A portion of the control-serum was heated to 60° C. for 10 minutes, and tested as before.

Result.—A good reaction, both microscopically and in sedimentation tubes, at 1 in 30 was obtained.

Are Dead Cultures Reliable for any Length of Time?—Using the control-serum, the following dead emulsions made in the laboratory were tested, dilution 1 in 30.

	Micro- scopical.	Sedi- mentation.
1. Agar emulsion of <i>Micrococcus melitensis</i> isolated at Haslar, heated to 65° for quarter of an hour, 0.5 per cent. formalin. Made 9.11.03	+	+
2. Agar emulsion from strain, given by Professor Wright, Netley. Made 8.11.04	+	+
3. Agar emulsion, from Haslar strain 2. Made 20.3.05	+	+
Control, normal blood	—	—

Thus, dead cultures made here more than two years ago were perfectly reliable, though the reaction is less rapid than when living ones are used.

Reliability of the Agglutination Reaction in Mediterranean Fever.—Here it may be stated at once, that in acute cases, I have found the reaction unmistakable, the serum in fairly high dilutions, acting on the *Micrococcus melitensis* almost immediately, clumping completely, and being generally easily visible with a 1-inch objective. With chronic cachectic cases of more than four months' duration, so commonly met with in Haslar Hospital, it is different, the reaction being often incomplete, slow, and only obtainable in very low dilutions, as shown by the following cases :—

1. J. W. Onset of the fever, April, 1904 ; returned to the Mediterranean in 1905 : immediate relapse. In November, 1905, the blood only agglutinated in dilutions of 1/10, yet the *Micrococcus melitensis* was in the same month isolated from his blood.

2. J. P. Onset April 19, 1905 ; now intense emaciation and neuritis. In November the blood agglutinated up to 1/10 ; with 1/5 the reaction was immediate.

3. T. S. Onset August, 1905 ; great emaciation and neuritis. In November the blood only reacted up to 1/10.

From these results, and from a great number of the same kind, I have formed the opinion that, when using the 1/30 dilution (if the technique is properly carried out), a positive agglutination reaction may be considered conclusive of Mediterranean Fever, past or present. On the other hand, it would not be correct to state that the patient is not suffering from Mediterranean Fever, when an examination of the blood gives a negative reaction with this dilution. I believe the chief sources of fallacy are—

1. Faulty technique ; incorrect dilutions, too long time, etc.
2. Faulty cultures ; containing false clumps before use, etc.
3. Faulty observations ; mistaking false clumps for true agglutination.
4. Faulty history ; the patient having previously had the disease.

The sedimentation test appears to me to be the least likely to give rise to errors, provided *clear* blood serum is used, and the emulsion be sufficiently strong to give a visible pellet at the bottom of the tube.

XIII. — REPORT ON THE PREVALENCE OF MEDITERRANEAN FEVER AMONGST BRITISH TROOPS IN MALTA, 1905.

By Lt.-Col. A. M. DAVIES, R.A.M.C., Member Mediterranean Fever Commission.

INTRODUCTORY.

On taking up the investigation of Mediterranean Fever in its epidemiological aspects, at the beginning of June, 1905, after consideration of the various lines on which such an inquiry might be best carried out, I became convinced that the most profitable course to adopt would be to devote the greater part of the time at my disposal to the study of the disease as it manifested itself amongst the British troops. Dr. R. W. Johnstone having made a general survey of the circumstances in regard to the whole population, civil, naval and military, up to the time of writing in the previous year, it seemed that a more detailed consideration of one branch of the subject—even though in numerical importance only a small one—might lead to useful results. I was the more inclined to take this course for two reasons: first, Dr. Johnstone had made such a comprehensive survey of the “sanitary circumstances and prevalence of Mediterranean Fever” in the previous year, that there was no need for another inquirer to go over the same ground a few months later; and, secondly, the fact that, in regard to the military population the statistical data—which are the foundations of an epidemiological inquiry—are to be relied on almost implicitly; while in regard to the population of Malta generally, our knowledge of the actual distribution of the disease, both in place and time, is at present so very imperfect, that the difficulties in the way of discovering the causative factors are extreme. Dr. Johnstone has mentioned this in his Report (p. 11). The notification of Mediterranean Fever throughout the population generally is extremely inaccurate, “only severe cases are notified, and not always these.” Now, whether in regard to differences of prevalence in different *places*, or variation in incidence at different *times*, unless there is good ground for trusting to the accuracy of the records of prevalence (*i.e.*, the notifications), the labour expended on inquiring into apparent outbreaks may be entirely thrown away.

The military population concerned is approximately 9000; if every case of Mediterranean Fever occurring in this population during even a single season were accurately recorded as to time and place of onset, and as to all the surrounding circumstances that could be

regarded as bearing on the problem, a body of facts ought to be forthcoming that would at any rate make some addition to our knowledge of the causation of the disease ; if not of the actual cause, at least of the conditions favouring the effectual operation of the cause. There seemed to be every likelihood, from the behaviour of the epidemic in May, that a large number of cases would occur amongst the troops during the hot season ; and that the amount of material for investigation would be large enough to make it justifiable to limit the inquiry to this particular line. From January 1 until September 30, 1905, there occurred 487 admissions for Mediterranean Fever from among the British troops, and it is to the study of this epidemic that I have chiefly given attention.

Three principal lines of investigation presented themselves : (1) It appeared to be necessary to make a detailed sanitary survey of the actual conditions under which the troops are living in the various barracks in the Maltese Command : I accordingly visited repeatedly every individual barrack, and examined into its situation, construction, water supply and drainage, as well as any other matters that seemed to need investigation. In the present state of our knowledge, or want of knowledge, as to the causation of Mediterranean Fever, it did not seem allowable to neglect any point of general sanitary importance, even though its connexion with the prevalence of this disease did not seem to be obvious. It does not, however, appear necessary to encumber this report with all the detailed results of this investigation, referring in many instances to somewhat minute points of sanitary engineering practice or military administrative procedure ; a brief summary only of the more important points is set forth in *Section I*, the details forming a separate Report presented to the Director-General, Army Medical Services.

(2) Concurrently with this the course of the epidemic was noted, and as far as possible inquired into at the time, and on the spot, week by week ; the intention being to record, as accurately as might be, the actual incidence of the disease in the various barracks and in the various regiments. As far as I have been able to ascertain, it has hitherto been the practice to allocate the cases of Mediterranean Fever to the barracks from which they have been admitted ; the object of this part of the special inquiry has been to trace the origin of these cases with greater exactness, ascertaining the movements of the patient previous to admission to hospital, and endeavouring to locate not merely the barrack, but the room, which he had been occupying for some time before admission. In this way it was hoped that some facts would be ascertained that would serve as indications as to the cause, or rather the mode of spread, of the disease. These results are summarised in *Section II* of this Report.

(3) As another means to the same end, the attempt was made to

interview personally every Mediterranean Fever patient, and elicit all the information procurable as to his habits, occupations and so on previous to being taken ill. Unfortunately some patients were too ill to be questioned, some were invalided before I was able to visit them ; so that from one cause or another not more than 187 were actually interviewed. I much regret that I was not able to carry this part of the inquiry further.

The information obtained by these three lines of inquiry is summarised in *Section III*, in which an attempt is made to correlate the various facts, and ascertain what relation exists, if any, between Mediterranean Fever prevalence and this or that sanitary condition. The conclusions arrived at, and certain recommendations submitted, are set down in *Section IV*.

Incubation Period.—A great difficulty, that has been experienced by all inquirers into the epidemiology of Mediterranean Fever, arises from the uncertainty that exists as to the length of the incubation period. Hughes, from his own experience, and from a study of the literature of the subject up to the time of writing (1897), considered that it might be "as short as three days in some cases. Probably 3 to 10 or 15 days is near the mark in cases where the first febrile onset is noted." Dr. Johnstone states that "the general impression amongst Maltese medical men seems to be that the usual incubation period is not more than 8 or 10 days." In five laboratory cases of human infection "in places where there was no prevalence of Mediterranean Fever, and no apparent source of infection other than in relation with infective material in the laboratory," the periods were respectively 5, 6, 8, 15 and 16 days. These infections appear to have all been by inoculation ; all were accidental, except the one with 16 days incubation, which was definite and purposeful. In the previous Reports of the Commission instances are recorded of infection by inoculation manifesting itself after 5 and 8 days in monkeys (Gilmour), after 6 days in goats (Shaw), after 6, 10 and 13 days in monkeys (Horrocks) ; in all these cases the agglutination test has been taken as the proof of infection. By the same test infection by feeding has been demonstrated in monkeys after about 24 to 32 days in several cases (Horrocks), and in one case, in a goat, apparently after 21 days (Horrocks). There is a sufficient agreement in these results to lead to the supposition (which is on other grounds reasonable) that with infection by inoculation the incubation period is shorter than by ingestion into the alimentary canal ; with inhalation of infected dust (monkeys) the incubation period is uncertain, 17 to 31 days or less (Horrocks).

How far the period of incubation observed in animal experiments can be considered to hold good in the case of man is doubtful ; the doses used in the laboratory have been enormous ; and as it would be

unreasonable to suppose that the quantitative element has no effect on the rapidity of development of the disease, the laboratory limits in all probability require to be considerably extended when the question of human infection in the ordinary way or ways has to be dealt with. As we are at present ignorant of the path of infection in man, we must assume that incubation may be as short as about a week, and may be as long as about five weeks, according as the infection is by inoculation or by feeding. But considering the very much smaller doses of pathogenic material likely to be actually absorbed than those used experimentally in the laboratory, it seems probable that not less than a fortnight should be regarded as a minimum period, and that the maximum period should be extended up to about six weeks at least.

In the absence of any more definite guidance I have adopted a fortnight as the most likely incubation period *at the least*, and a further fortnight as *probably* needful to be allowed, for incubation. That is to say, if a man moves from Barrack A to Barrack B, and subsequently develops Mediterranean Fever, I consider that if his illness commences within a fortnight of his change of residence, the infection was *almost certainly* contracted at A; while if it commences within a month of the move, it has *more probably* been contracted at A than at B. I have not been able to ascertain any shorter instances of incubation than the two quoted by Dr. Johnstone (*Report*, Part II, p. 15), of 8 and 11 days respectively; and the behaviour of the epidemic in the Essex Regiment (detailed in *Section II*) points to about five weeks as probably the longest usual interval between inspection and onset of illness. Further observations are much needed in regard to this matter.

Diagnosis.—All cases admitted to the military hospitals in Malta, that have been returned as Mediterranean Fever, have been diagnosed as such, both by their clinical features, and by the results of the agglutination test; this has been invariably applied, and no case has been returned as Mediterranean Fever unless the reaction has been definite and complete.

SECTION I.

The most important points in regard to the sanitary condition of the barracks in Malta may be shortly summarised under the general headings of situation, construction, water supply, and drainage.

In Valletta itself, and Floriana, there are seven separate barracks, all more or less antiquated in their plan and construction.

In *Lower St. Elmo* an infantry battalion (2nd Essex Regiment until July, 1905, then 1st Lancashire Fusiliers) is accommodated in a part of the fortress that occupies the extremity of the promontory

separating Marsamuscetto or Quarantine Harbour from the Grand Harbour.

The fort adjoins the sea on two sides, but, being excavated in the rock, the barracks are entirely deprived of any advantage from this proximity; while on the south they adjoin the most densely inhabited part of the city, and on the east are shut in by the more elevated part of the fort, called Upper St. Elmo. The barrack rooms, 52 feet in length, are casemates arranged in two tiers, and are very imperfectly ventilated; they accommodate 23 men in each, and the cubic space is barely 600 feet per head. The drinking water supply is ample; a second quality of water is provided for ablution, not always in quite sufficient quantity; sea water is laid on for flushing purposes. The latrines are water-closets of good pattern, and have recently been fitted with new automatic flushing apparatus; the urinals are of the ordinary type, having a scanty flush of water, and being also treated with a coating of kerosine oil. The drainage has been remodelled in recent years, and is satisfactory; the soil pipes are ventilated, and accessible inspection chambers provided at junctions of the underground drains, which discharge into the Civil Government sewer.

The water supply and drainage arrangements of these barracks are in the main satisfactory; their construction is very insanitary, the ventilation bad, and the cubic space insufficient. Although it is the practice in Malta generally to issue tentage during the summer months, sufficient to allow of 25 per cent. of the troops sleeping out of the barrack rooms; since June, 1903, Lower St. Elmo has been excepted from this privilege, for reasons which I have not been able to ascertain. These barracks are, in my opinion, more in need of this thinning-out process than any others in the whole island. The accessories are fairly satisfactory, except that one of the cookhouses adjoins a stable.

Upper St. Elmo adjoins the last mentioned on the east, is at a higher level, and is in every way more advantageously situated, being freely exposed either to the sea or the harbour on three sides. The barracks are occupied by two companies of Royal Garrison Artillery; but, the quarters being insufficient for their accommodation, many of the men live and sleep in tents. A lower portion, consisting of two tiers of small casemate rooms, is occupied by 96th Company R.G.A.; the rooms, being only about 25 feet long, are not difficult to ventilate, but the cubic space allowed (440 to 536 cubic feet per head) is very small. The upper portion consists of rooms of more modern construction, and not of casemate shape; but the cubic space, about 550 cubic feet per head, cannot be considered as sufficient. The water supply is satisfactory. The latrines are not satisfactory; the lower latrine, used by 96th Company, has recently been fitted with an automatic

flush, but it is in a dark and cramped situation ; the upper latrine, used by 65th Company, was in a bad state at the time of my visit, the flushing arrangement being completely out of order, and the pans full of excreta. The urinals are scantily flushed ; oil has not been taken into use. The drains are generally in a satisfactory condition, accessible inspection chambers being provided where required ; some points of detail need attention in regard to the drainage of the new sergeants' mess.

The barracks generally are better than Lower St. Elmo ; but the continual use of tents in this very confined situation must lead to fouling of the ground.

St. James Cavalier is a small barrack, accommodating a detachment of 138 men belonging to the Royal Garrison Artillery stationed in Upper St. Elmo, at present 65th Company. It is situated in the upper part of Valletta, the barrack rooms being casemates similar to those in Lower St. Elmo ; two of the rooms are 51 feet in length, with most inadequate openings for ventilation ; they are, however, authorised to accommodate 32 men in each, giving a cubic space of only 535 feet per head, which is quite insufficient ; four other rooms are not so long, and therefore not so hard to ventilate ; all the six rooms are authorised to hold more men than there is actually space to accommodate, unless the bed-cots are arranged in three rows, a practice which would be most insanitary, and is universally condemned. At least 750 cubic feet should be allowed per head ; and, even with this increase, it is doubtful if No. 6 room would be reasonably fit for occupation. The drinking water supply is satisfactory, also that for ablution ; but for latrine flushing it has had to be carried up by hand. Throughout the greater part of the past summer all the water supply for the latrine has had to be carried up by hand, as a regimental fatigue, the result being that a minimum quantity has been provided, and the condition of the latrine has been insanitary. It is absolutely necessary that water should be laid on to a latrine in a permanent barrack. With this exception the drainage arrangements are satisfactory. The situation is bad, and the construction of the barracks insanitary.

Floriana Barracks, including Salvatore Counter Guard and Notre Dame Ravelin, are occupied by a battalion of infantry, at present the 1st Royal West Kent Regiment. They are situated on the north side of Floriana, and within the outer line of the landward defences of Valletta, of which they form a part. The *old* portion of these barracks consists of a range of 12 casemate rooms, about 80 feet in length, each accommodating 30 men, with an allowance of from 700 to 1203 cubic feet per head. In casemates of this length, with no window openings except at the two ends, it is impossible to secure proper ventilation. At present the barracks are not crowded, as the

accommodation is sufficient for 920, and the battalion does not number more than 780. A peculiarity of these barracks lies in the circumstance that the Malta Civil General Hospital occupies the upper part of a building, on the ground floor of which are the regimental offices, stores, &c. There is no communication between the ground and upper floors, and the drainage of the hospital is quite distinct from that of the barracks, but it is most undesirable that such a building, into which infectious cases (*e.g.*, possibly cholera or plague-stricken sailors) might be admitted, should form part of a structure occupied as a British barrack.

The *New Barracks* consists of three block of two-storey buildings, each accommodating one company, that have only recently been completed; the rooms are well arranged, according to modern principles, each holding 26 men, with an allowance of 755 cubic feet per head.

The rooms in *Salvatore Counter Guard* are small casemates with no through ventilation at all, and with a scarp wall distant only 12 yards from the front of the rooms; the movement of air must be very limited at any time, and adequate ventilation impossible; in spite of this, the allowance of cubic space is less than 600 feet per head; neither have the men been thinned out at night during the hot weather.

Notre Dame Ravelin consists of a range of 16 small rooms on the ground floor, accommodating 5 men in each, with an allowance of 900 cubic feet per head; and of seven huts, each for 18 men, with 600 cubic feet per head. These are all well ventilated, and of satisfactory construction. The huts stand on a concrete platform, and are slightly raised from the ground.

There is a good supply of No. 1 water for drinking, and No. 2 water for ablution purposes; for flushing the supply (No. 2) is sometimes defective in the Old Barracks; a larger tank and separate supply for the latrine seem to be required. The drainage of all these barracks is of modern construction, and, in the main, satisfactory. One of the latrines in the New Barracks was in bad order at the time of my visit, partly owing to a structural defect; and in several places the internal surface of the drains is uneven, leading to obstruction, or retardation of flow; gully gratings are in some places deficient. The urinals are treated with oil, and also flushed with water.

Of these barracks it may be said that the New Blocks and Notre Dame Ravelin are satisfactory, but that the Old Barracks and Salvatore Counter Guard are bad, and incapable of being made suitable for accommodating troops. Great complaints are made of the extensive fouling of the ground in the neighbourhood of these barracks, where a large number of Maltese labourers are employed in road-making, etc. The troops have no control over these people, and the civil authorities appear to be powerless in the matter.

St. Francis Barracks, Floriana, are a small range of barracks of very old type, partly on one, partly in two stories, occupied by a company of Royal Engineers. Nos. 3 and 4 rooms on the ground floor are large apartments, 66 × 29 feet, authorised to accommodate 45 men in each; the ceiling is arched, and, if the height be reckoned as 26 feet, the cubic space per head amounts to 1079 cubic feet, as shown in the Barrack Return; but if 12 feet of height only be reckoned (according to the general rules for calculating cubic space), the amount per head is only 500 feet. The means of ventilation are insufficient. Nos. 6 and 7 are large rooms on the upper floor, fairly well provided with windows and ventilating openings, but difficult to ventilate, on account of their excessive width, 40 feet. These are very unsatisfactory barracks; if they are to continue to be occupied, a cubic space of 750 feet should be allowed, and no greater height than 12 feet should be reckoned as available for ventilation purposes. The water supply is satisfactory. The latrine and urinal are of very old pattern, and require reconstruction; the drainage is modern, and in good order.

Marsamuscetto Barracks, occupied by the Army Ordnance Corps, consist of two rooms on the ground floor, each accommodating 41 men; the rooms are arched casemates, 72 feet in length, having windows only at one end; adequate ventilation is impossible, yet the effective cubic space (reckoning a height of 12 feet) is only 540 feet per head. The number of actual occupants is at present less than half the allotted number, so that there is no overcrowding; but the building is unsatisfactory. The latrine is within 20 feet of the cook-house; it is flushed only twice a day; a third flush at least is required. The urinal is intermittently and scantily flushed with water. The drainage is in good order.

The *Old Laboratory Barracks*, occupied by Army Service Corps, Army Pay Corps, Military Foot Police and Garrison Staff, consist of four rooms at an upper level, and two at a lower level; the upper ones are arched casemates, with very inadequate ventilation; the lower ones are in a very cramped and confined situation. The latrine is exceedingly cramped, dark, and ill-ventilated. The barracks are said to be condemned. They are incapable of being made really satisfactory from a sanitary point of view. The water supply and drainage are inadequate.

Manoel Fort and Hutments together accommodate an infantry battalion; up to the beginning of June they were occupied by the 1st Rifle Brigade, since then by two companies of the Lancashire Fusiliers. The situation, on a small island in Marsamuscetto Harbour, is favourable, with free air space all round. Seven of the barrack rooms in the fort are casemates, about 32 feet in length, each accommodating nine men, with 600 cubic feet per head; being small rooms their ventilation would not be unsatisfactory, but that the blank wall

of the chapel and officers' quarters is only a few feet distant from the front of the rooms, and materially interferes with the free passage of air. Three other rooms, accommodating 36 men in all, are free from this disadvantage. The hutments consist of 28 huts, each accommodating 18 men; they are well raised from the ground on pillars; the surface beneath is cemented, clean, and dry, and they are not overcrowded. The water supply is satisfactory; No. 1 water is used for drinking and washing in the hutments; in the fort, No. 2 water is collected in tanks in the rainy season, and used for ablution and flushing purposes. The latrines are all on the dry earth system; a double set of buckets is provided, but the excreta are removed only once a day, in the early morning. The water drainage system takes urine and ablution water, and discharges direct into Marsamuscetto Harbour; it is of modern construction, and satisfactory. Except for the retention of the dry earth system, these barracks are in a good sanitary condition. A connexion should be made with the Civil Government sewer as soon as possible.

Tigne Barracks, occupied by three companies of Royal Garrison Artillery, consist of the old fort, two new blocks of quarters, and 15 huts; in addition are married quarters, offices, institutes, &c., all of modern construction. The situation is excellent, having the open sea to the north and east, and Marsamuscetto Harbour to the south. The fort contains only a few small rooms; one on the upper floor is well ventilated; seven on the ground floor are at a lower level surrounded by the fort wall, and badly ventilated; only two are in present occupation, and all (it is said) will be evacuated shortly. The two new blocks, each accommodating 100 men, are two stories in height, and satisfactory in every way; except that the urine tubs have to be carried *through* the rooms on the upper floor from the verandah at back to the staircase in front, thereby leading to fouling of the floor with possibly infective material. The huts accommodate 18 men in each, are well raised off the ground, which is concreted and easily kept clean, and are not overcrowded. In the summer 25 per cent. of the men sleep in tents; in 99th Company no trestles or bed boards were supplied, and the men's mattresses were placed on the ground; this should not be allowed.

At present No. 1 water is used for all purposes, 20 gallons per head being allowed for everything. Until recently the latrines were on the dry earth system; now that a water latrine has been erected, it will probably be necessary to draw on the rain-water tank beneath the barrack square; but it would be better to lay on a supply of flushing water. In the fort the ablution water and urinals drain into a system that eventually enters the open sea; a dry earth latrine remains in use, which is regrettable. For the rest of the barracks an excellent modern drainage system has just been completed, discharging into the

Civil Government sewer. Two dry earth latrines still remain in the western part of the barracks, one being for the use of the school ; the other is no longer required ; this should be closed, and a water latrine provided for school use. A large new latrine has just been opened to the north-east of the barrack square, containing 34 seats ; this is flushed three times daily, at present with No. 1 water. It is an important question, affecting several barracks, whether this No. 1 water, the supply of which is very limited, should be used for flushing purposes. There is great danger of the quantity being restricted, leading to an offensive and insanitary condition of the latrines ; it is also very doubtful whether its use for this purpose is justifiable under the circumstances obtaining in Malta. I am very strongly of opinion that a supply of flushing water should be laid on to all latrines, and used in great abundance ; and that No. 1 water should only be used (as a rule) for drinking and cooking purposes. In the present case the greatest care should be taken to prevent this new latrine, connected with a new system of drainage, from degenerating into the filthy and dangerous state that so many of the latrines in Malta have been allowed to get into, principally through deficiency of a proper supply of water, in some instances unavoidable, in other instances the result of neglect.

The urinals are treated with paraffin oil and lampblack, and water flushing is used as well. The new urinal contains 26 stalls, the authorised allowance (4 per cent.) for the number of troops occupying the barracks. It is extremely inconvenient to collect all the urinals together in one place ; and when so many stalls are provided in one range most of them are not used ; not more than six or eight stalls are ever required in one range ; the rest are useless, and lead to a great waste of water.

Except for the minor points of detail, these barracks are satisfactory in every way as regards situation, construction, water supply, and drainage ; but care must be taken in regard to the matter just mentioned in order to maintain this satisfactory condition ; if water is stinted for flushing purposes, the state of things will be very different.

To the south-east of the Grand Harbour, and elevated a considerable height above the sea level, lie the Verdala Barracks, a chain of fortifications called the Cottonera Lines, and at the harbour's mouth Fort Ricasoli.

Verdala Barracks, occupied by an infantry battalion (2nd Hants), consists of 66 small casemate rooms, each about 25 feet in length, and accommodating 10 men ; they are disposed on two floors and in two rows ; being small rooms, and in a fairly airy situation, their ventilation presents little difficulty ; the cubic space allowed is, however, only 515 feet per head, which is not sufficient ; the accoutrement shelves are fixed to the walls in a continuous line, and the bed-

cots are only 12 inches apart from each other. Drinking water is laid on, and the supply is ample; for washing, No. 2 water is pumped up by regimental fatigue; until July, 1905, all the water required for flushing purposes was also similarly pumped up; now, however, salt water is laid on for this purpose, but the supply at the time of my visits was uncertain, and frequently No. 2 water had to be pumped up, regimentally, as before. The regimental latrine is situated rather near the cookhouse; it is of Jennings' continuous pipe pattern, and until recently was only flushed twice a day; it is now flushed three times daily, at 9.0, 2.0, and 5.0, and this is hardly sufficient. When a proper supply of salt water for flushing is available, it should be done four times a day. The urinal has 14 stalls, aggregated together, many of them being never used; both water flushing and oil application have been practised, the former ineffectually; the stone floor is very uneven and requires putting in order. The underground drainage is modern and, on the whole, satisfactory. Some additional ventilation to the system would be advisable, and provision for automatic flushing instead of the present inefficient method of flushing by hand with barrels of salt water, when it is available. The sanitation of these barracks has been well looked after, under circumstances of considerable difficulty.

The quarters which together make up the *Cottonera Lines* are, St. Clement's Bastion, Zeitun Gate, Polverista, St. John's and St. Paul's Bastions, Couvre Porte, Vittoriosa, Fort Salvatore, Zabbar Gate, and Notre Dame; accommodating in all about 780 men, that is, an infantry battalion. In the early part of 1905 they were occupied by the Royal Sussex, and then by the Lancashire Fusiliers; since the departure of this regiment for Lower St. Elmo they have been mostly vacant, except for detachments of the Hants Regiment in Polverista and St. Clements. All of the barracks are old and defective in many ways; proper ventilation is very difficult; if their occupation is continued, at least 750 cubic feet per head should be allowed, reckoning only a height of 12 feet as of any value for ventilation purposes. The small rooms at Zeitun Gate are fairly sanitary; but the larger ones, Nos. 5, 6, and 7, measuring about 32 x 20 feet, without any windows except in the front wall, are impossible to ventilate satisfactorily. The rooms in Polverista, which are arched casemates, 33 feet long, accommodating 14 men in each, are also very inadequately ventilated. The small rooms in St. John's and St. Paul's, although inconvenient, are not difficult to ventilate. At Couvre Porte, No. 11 room has no window at all, and is unfit for occupation. Vittoriosa has three large rooms, each accommodating 34 men, which are airy and well lighted, though a proper cross ventilation is not possible. The three large rooms at Fort Salvatore, each measuring about 80 x 20 feet, cannot be adequately ventilated by the very small openings that at present exist. At

Zabbar Gate the two large rooms, though light and airy in appearance, are very hard to ventilate, on account of their great width, 36 feet. Notre Dame, consisting of eight small rooms, is fairly satisfactory.

The water supply of these small barracks is a matter of some difficulty, and in connexion with the latrine arrangements requires more attention than has hitherto been given to it. No. 1 water for drinking is laid on, and is sufficient and always available. For washing purposes and for flushing latrines and drains No. 2 (collected rain-water) has, until last July, had to be pumped up by regimental fatigues; at Polverista the pump has broken down several times (twice during the time I was making my visits to the barracks in July and August); the labour of working this pump appears to be excessive. I was informed that a fatigue of nine men, working six hours a day, was required. On three occasions I found the latrine empty of water, but fouled with excreta; this appeared to be a not uncommon occurrence. It has been the same with the other outlying barracks. Salt-water flushing is in course of being provided, but so far the supply has been uncertain. Until an ample supply of water is available for adequately flushing the latrines—at least three times a day—and for keeping the extensive system of drains in good order, these barracks are not fit for occupation.

The drainage generally is modern and satisfactory in construction. There has been considerable complaint of bad smells in front of Polverista; the drains are properly constructed, but more water is required for flushing the verandah drain and down pipe leading into the collecting drain below.

Fort Ricasoli lies at the mouth of the Grand Harbour, on its eastern side, in an ideal situation; it is open to the Mediterranean and the harbour in three directions, and has the open country to the east, with no villages near. An ample supply of drinking water is laid on, used also for ablution purposes, and salt water is drawn from the sea for drain flushing, by a pump independent of any other supply. The drainage passes direct into the sea, by three independent systems of drains, which have been laid down within the last few years according to modern principles, and which are in good order. Some additional provision of fresh air inlets would, in my opinion, be desirable. The latrines and urinals are well kept, a mixture of tar and kerosine oil being used for the latter; one latrine was found to be without any water (but full of excreta) on one occasion, owing (so I was informed) to choking of the branch supply pipe; even under the quite exceptionally favourable conditions as regards water supply at Ricasoli, strict supervision and watchfulness are necessary.

The barrack rooms are mostly large and lofty, having plenty of window space on one side (facing the square), but no openings on the other (which is the outside of the fort); five such rooms are each over

100 feet in length by 22 feet wide, accommodating between 50 and 60 men in each; being about 23 feet in height, the cubic space per head (about 1100 feet) is large; but it is not all available for ventilation purposes, not more than 12 feet of height being really effective; on account of this height and the width of the rooms, it is difficult to get a free change of air. The bed-cots are placed very close together. No. 1 room, 80×22 feet, has two windows only on one side, and a doorway at one end; there are no windows on the other side or at the far end, which is quite unventilated.

The actual barrack accommodation is for 480; but three companies Royal Garrison Artillery are normally stationed here, with a strength of about 700; about 120 are quartered in outlying forts, and about 150 in tents pitched in the barrack square, occupied all the year round. In the summer 25 per cent. extra tent accommodation is drawn. Although the construction of the barracks is not sanitarily satisfactory, the general good hygienic conditions of Ricasoli, and its fine airy situation, should make it a healthy station.

Outlying Forts on the eastern side:—Small bodies of men are accommodated in several small forts in this direction. In every case the cubic space is sufficient, though, from military exigencies, ventilation is restricted; drinking water is laid on to all the forts; but for some months during the past summer this pipe supply has been cut off, and the water has been carried out to the forts in barrels. The drainage arrangements are generally satisfactory, as regards slop water and urine; dry earth latrines are in use. These require to be more carefully supervised, and the removal should be more frequent.

The barracks hitherto mentioned have been, in the main, old buildings, dating from a pre-sanitary era, though added to from time to time, and with drainage and water supply modernised more or less efficiently. On the north side of the island are two extensive ranges of barracks, one of which, St. George's, was built in 1860, and has since been added to, and the other, St. Andrew's, has only been completed in the year 1905. Each of these accommodates an infantry battalion.

St. George's Barracks, occupied by the Royal Dublin Fusiliers, consist principally of single-storey blocks of small barrack rooms, accommodating 13 men in each, with 605 cubic feet per head; these are of good construction, and very fairly well ventilated; the accoutrement shelves are fixed to the walls in a continuous line, and the bed-cots are only 12 inches apart from each other, which causes what may be called an artificial overcrowding at night. There are two new double-storey blocks of quite modern design, airy, well ventilated, and well arranged; the rooms accommodate 16 or 18 men in each, with an allowance of 750 cubic feet per head. A defect in the arrangements is that the urine tubs have to be carried through the

rooms on the upper floor, from the back to the front (as at Tigne), thereby leading to fouling of the floor with urine, which is most undesirable.

No. 1 water is laid on for drinking and also for washing, the supply being quite ample; it is also laid on to the married quarters for flushing purposes as well. In the barracks sea water is pumped up for latrine and drain flushing, but owing to defects in the pumping arrangements, the quantity of water provided has been insufficient, and the latrines have not been properly cared for. The drainage is of modern construction throughout, and is well looked after; the latrines are flushed three times a day if water is available; the urinals are in good order, a mixture of lampblack and kerosine being applied. There are several minor defects, which might be easily rectified; one frequent source of drain obstruction in Malta is the readiness with which sand and gravel are blown into and washed into and through gullies; in these barracks, which are well exposed to the wind, this occurs to a considerable extent, and causes some difficulty in keeping the drains clear; raised parapets, to keep out surface washings, and deep traps might be supplied in some places with advantage.

St. Andrew's Barracks were only completed in the early part of 1905, and were taken over by the 1st Battalion Rifle Brigade in June. They consist of nine double-storied company blocks, the rooms accommodating 14 men in each, with a cubic space of 800 feet per head. They are satisfactory in every detail, except for the necessity of carrying urine tubs through the rooms on the upper floors. The water supply is No. 1 for all purposes. The drainage is satisfactory in its main features, but there are several points of detail that require attention, such as the provision of accessible manhole covers (instead of cemented slabs), easing off of right-angled junctions, etc.

Pembroke Camp is a musketry camp near St. Andrew's Barracks, occupied by parties of men from various regiments in succession throughout the whole year, as many as 800 or 900 being sometimes under canvas at once. Its sanitary condition is very unsatisfactory. The ground is rocky and uneven, and difficult to keep clean; the sites of the tents are never, or hardly ever, changed. There is one dry earth latrine of 26 seats for the whole camp; this is not sufficient accommodation for the numbers that are frequently present; the latrine seats are badly constructed, being too high (or the pails placed too low); fouling of the ground with urine results. The pails are removed only once a day, between 4 and 5 a.m., the result being that for the greater part of the 24 hours the air of the camp is fouled by excretal emanations; flies are also attracted in great numbers. The woodwork of the latrine is in bad repair. The urinal consists of a

plain marble slab like a native convenience; it is flushed with water and no oil has been applied. At the north-west end of the camp is a cesspit, connected with the officers' w.c., apparently unventilated, and in close proximity to the water tank and officers' cookhouse. A water drainage system is now being carried out, and this cesspit should be removed.

Pembroke Camp is in a very bad sanitary state, not due to any want of care on the part of the camp authorities, but on account of obvious defects of design and construction in what may be called minor details. A small *permanent* sanitary staff should be provided to keep the camp in as sanitary a condition as may be possible, and lessen the difficulties resulting from the constantly shifting character of the population.

In regard to the *Outlying Forts* in the Western District the same remarks apply as to those in the Eastern, except that No. 1 water is laid on in each case, and is ample in quantity. The dry earth system is in use, and is fairly satisfactory, except at Maddalena, where the accommodation is insufficient, there being only two latrine seats. Everywhere removal only takes place once a day, which is not enough. A modern drainage system for slop water, &c., has been laid down in each case.

Imtarfa Barracks consist of four large blocks, accommodating 233 in each, and four smaller blocks, accommodating 110 in each, all of two stories; the rooms are constructed for either 16, 18, or 20 men, with a space of 750 cubic feet per head. They are excellent barrack rooms in every way, well built, and with every convenience. No. 1 water is laid on for drinking and washing; rainwater is collected in underground tanks, and pumped up regimentally for cleaning and flushing purposes. The latrines have hitherto been on the dry earth system, but a water carriage system will be introduced very shortly. A complete system of drainage has been constructed, to which the latrines can be readily connected up. The dry earth latrines were in a satisfactory condition at the times of my visits, but I was informed that this had not been the case earlier in the summer, and that it had been found necessary to employ regimental fatigues to apply the dry earth thoroughly. It is difficult to get the dry earth system properly carried out anywhere (though principally a matter of regimental discipline), but the difficulty is much increased in the case of a body of men who have been accustomed to the use of water latrines, that require no attention on the part of the individual. In the present case a rather considerable prevalence of enteric fever has been due, in all probability, to faulty carrying out of the dry earth method at Imtarfa. The latrines are emptied by contract once only in the 24 hours, about 2 A.M.; during the greater part of the day, therefore, they are full of excreta, and the air of the barracks proportionately fouled; flies are

quite a plague in some parts of the lines, a fact which is always significant and generally of ill omen. The urinals are treated with a mixture of colza oil and tar ; this has acted most satisfactorily, applied once a week ; the urinals here were in a better state than any others in Malta at the times of my visits. The underground drainage system is satisfactory on the whole ; at one or two places the fall appeared to be hardly sufficient, *e.g.*, at the north-west corner of the canteen a considerable amount of deposit was found ; there was also a good deal of deposit in the main collecting drain at the east end of the barracks, north of the junction with No. 1 cookhouse drain ; in both places this has, I understand, occurred before, more than once. Additional flushing is required, and careful supervision to see that no stoppage takes place.

Some surface drains which take the washings of the verandahs of married quarters lead into the foul drainage system, passing through a gully trap to cut off the foul air. Such is the case in M, N, O, and P Blocks, Married Quarters. But the verandahs of these blocks are not habitually, and probably very seldom, if ever, washed down with water ; consequently in dry weather no water gets into this gully, or at least not enough to provide an efficient seal. The trap was unsealed at the time of my visit, in the case of each of the above-mentioned blocks, the trap being almost dry, and choked with sand, which at the bottom was moist and foul-smelling. These traps require to be seen to, and filled with water periodically. The sewage is at present conveyed to a kind of septic tank, the effluent from which is applied to land in the Kleir Wied, to the north of the barracks. This method of disposal is quite inoffensive.

The situation of these barracks is all that could be desired. They stand on an isolated hill, some 600 feet above the sea, exposed to the fresh air on all sides, and with no insanitary dwellings near at hand. The barracks are well constructed and sanitary ; with a good water supply, and a proper system of sewerage and refuse removal, the troops should be free from all epidemic disease. It has, however, unhappily been the case that there has been a good deal of sickness this past year, due to preventable causes.

At Ghain Tuffieha and Mellieha, in the extreme west of the island, are camps used by the troops, chiefly during the winter season ; also at Ghain Tuffieha is the standing camp of the Mounted Infantry, the permanent strength of which averages 250 to 300 men throughout the year. The situation of each of these camps is quite satisfactory. In each case there is a good and ample supply of drinking water laid on ; also a drainage system on modern principles. At Mellieha the latrines are water latrines, and the whole of the drainage is conducted to a small septic tank, the effluent from which passes into the open sea. At Ghain Tuffieha, up to the present, the dry earth system has been in

use. The drains carry off drainage from cookhouse, stables, urinals, etc., to a septic tank, hermetically sealed up with great care, the effluent from which passes into the sea. When water latrines have been provided, in place of the dry earth buckets, and connected with the existing drains, this camp ought to be extremely healthy, provided the ordinary rules of camp sanitation are strictly carried out, and the drains carefully looked after.

Fort Chambray, Gozo, is an old fortress of the Knights, in which there is accommodation for (nominally) 400 troops. The barrack rooms, four on the ground floor and four on the upper floor, are 100 x 20 feet, with good windows at each end, but no openings, except a doorway, at the sides. They are therefore very difficult to ventilate. The accoutrement shelves are fixed to the walls, touching each other, and the bed-cots are very close together; but as only one company is at present in occupation there is no overcrowding. Drinking water of good quality is laid on from the public supply. Collected rainwater is pumped up regimentally for washing and flushing purposes. The drainage system is partly modern and partly old, but is now nearly all remodelled. On the whole it is satisfactory. A foul catchpit outside the married men's latrine, and a series of deeply-sunk silt traps in rear of the married quarters require certain obvious and easily practicable alterations. The latrines are on the dry earth system, with removal once a day only. Urinals are treated with lampblack and oil. These barracks are admirably situated for health, and are satisfactory in all important particulars.

Hospitals.

Valletta Military Hospital contains 232 beds, and also has quarters for 65 non-commissioned officers and men of the Royal Army Medical Corps. The buildings are ancient, and not well adapted for hospital purposes according to modern requirements. The situation is unfavourable, as, although it borders on the Grand Harbour to the east, on the west and south it is closely surrounded by crowded dwellings of the poorer class; moreover, the principal wards are deprived of the beneficial effects of the cool north-west wind by reason of the lofty houses built on higher ground in that direction. The wards are lofty, and, on account of the thickness of the walls, cool in summer and warm in winter.

The principal feature in the hospital is the famous "Long Ward," probably the longest room in the world, being 503 feet in internal length, without any break in the continuity of the ceiling or east wall. Its width is 35 feet, and its height 32½ feet. Near the middle a transept is given off to the west, of nearly equal width and height, and about 100 feet in length, forming part of the same chamber. To facilitate administration the whole apartment is divided by partitions,

10 feet high, into northern, southern, central, and western portions (20A, 20B, 20, and 20C); but from a sanitary point of view it is all one chamber. In 20A are accommodated 50 patients, chiefly Mediterranean and enteric fevers; in 20B are 60, venereal and slight cases; in 20 and 20C are slight fever cases. The cubic space is very large, 4000 cubic feet per head, reckoning the whole height of $32\frac{1}{2}$ feet; if the height be taken as 12 feet, it is over 1500 cubic feet per head. These amounts appear to be ample. There are difficulties in ventilation, however, in spite of this ample cubic space, which, indeed, is of no advantage if it interferes with the free access of external, and exit of internal air. It is obviously more difficult to change the air of a room 30 feet wide than that of a room 10 feet wide, the amount of window space being the same in each case. In this instance the width is 35 feet, and the window space is not large. There are very few windows in the lower part of the walls. In the upper part there are plenty; but there is reason to believe that they have not been opened, and kept open, so freely as would have been desirable, and that consequently the ventilation of this large apartment has not been satisfactory. Notwithstanding its coolness and spaciousness, the difficulties in maintaining purity of the air, and the impossibility of isolating the patients, render this "Long Ward" an undesirable place in which to treat the sick, although at first sight to be very well adapted to the purpose. The wards on the upper floor are of moderate size and well ventilated. The flooring of the wards is of cement concrete, having a smooth impermeable surface that is easy to keep clean.

The water supply is good and ample for all purposes. The drainage system has been entirely reconstructed within the last few years, and is in accordance with modern requirements; the drains discharge into the civil sewers at three different points, being cut off by proper disconnecting arrangements. Accessible inspection chambers are provided freely. A few points of detail in construction require attention; as, *e.g.*, a proper grease trap for the cookhouse; abolition of the large foul catchpit near south-east corner of Lower Square. Other important requirements are (1) a new latrine for 20B Ward, the present one being in bad repair; (2) concreting the rough floor of latrine and urinal for No. 37 Ward.

The Families Hospital, though in rather a cramped situation, is fairly satisfactory.

The R.A.M.C. barrack room is a large apartment, 96×31 feet, with an annexe on one side $28 \times 17\frac{1}{2}$ feet; it is well lighted and airy in appearance, but on account of the great width and absence of cross ventilation it is difficult to secure a proper purity of the air.

Cottonera Hospital is a modern building of good general design, and is in an excellent, airy, and healthy situation, standing in its own grounds, at a considerable elevation. It has four large wards,

128 x 26 feet, of 32 beds each, and several smaller ones, 156 patients being accommodated in all. The wards are well designed, well lighted, and well ventilated. The ward annexes are capable of improvement. The principal sanitary defect in this hospital lies in the material of the ward floors, which are made of a soft and easily friable porous white stone; it wears away unevenly into holes, which are difficult to keep clean; the operation of ward-sweeping twice a day fills the air of the ward with fine dust, which is afterwards deposited on the patients, on their beds, and on any articles of food that may be exposed; a good deal of it must be inhaled. The floor spaces between the beds have been treated with some hardening preparation that makes the stone impermeable, but the main part of the floor in the centre has not been so treated. The provision of a smooth impermeable floor, as at Valletta, is an urgent necessity. Water supply and drainage are quite satisfactory.

Forrest Hospital (31 beds) is a hired house, not designed for a hospital, but in as satisfactory a condition as can be expected. The water supply and drainage arrangements are in good order. A considerable number of the patients (20 to 30) are treated in tents all the year round (owing to want of sufficient accommodation), which greatly adds to the difficulties of maintaining a good sanitary condition of the hospital and its accessories.

Imtarfa Hospital (42 beds) is a new building constructed in accordance with modern principles, and is in every respect satisfactory.

Citta Vecchia Sanitarium (80 beds), is an old palace of the Knights, with large airy rooms, and well fitted for treating convalescent cases; the water supply and drainage are satisfactory.

Gozo Hospital contains 15 beds, and is satisfactory in its situation and construction, water supply and drainage arrangements.

Married Quarters.

There is accommodation for about 650 married families in the Maltese garrison, and in the great majority of instances this accommodation is remarkably good. In this category are to be placed the two new blocks at Floriana, known as Misida Bastion (44 quarters), the new block in St. Francis Ravelin (6), the four new blocks at Tigne (65), D Block, St. George's (10), all St. Andrew's (36), Verdala New Block (24), Ricasoli new blocks (18), and all Imtarfa (55). These quarters are all excellent in every way, airy, and well ventilated, the water supply laid on, and good water-closets of modern wash-down pattern provided; in nearly every case also the situation is good; they are some of the most agreeable residences in the island. There are certain minor defects in sanitary detail that require attention, but nothing to interfere with their permanent usefulness, healthiness, or convenience.

The largest block of married quarters in Valletta is the building known as the Camerata, facing the Valletta Military Hospital; this accommodates 92 families, and is generally nearly full. It is an old building of six stories, and is comparable to a block of artisans' dwellings in London; being situated in the middle of the town, it is not so fresh and airy as other quarters, and some of the rooms are without direct communication with the external air; new closets of first rate pattern have been recently supplied on each floor, and the building is kept in admirably clean and good order; so that, in spite of its being somewhat crowded it is really quite a sanitary and satisfactory block of dwellings.

The other older quarters, such as those in Upper and Lower St. Elmo, Floriana Pavilion (14), St. Francis Ravelin (16), Fort Manoel, the old blocks at St George's (55), the old quarters at Ricasoli (20); St. Nicholas and Gozo (21), though not so convenient, or so well off in the way of closet accommodation and water supply, are nevertheless in a fairly good sanitary condition.

The large block of hired quarters in Strada Magazzini, Floriana, has been put into as good a condition as is practicable in regard to water supply and sanitary arrangements; but there are certain grave defects of construction in regard to the drainage (faulty pattern of w.c. liable to become untrapped, ill-ventilated closet chambers, inferior work in the underground drains) that prevent their being considered satisfactory quarters; they resemble the ordinary private houses in Valletta, etc., and are not to be compared to any of the newly erected married quarters that have been just mentioned. The hired quarters at Tigne are to be placed in the same category; those in Strada Capuccini, Floriana, are satisfactory.

There are some married quarters on the Cottonera side (Fort Salvatore (14), Vittoriosa (3), and St. Nicholas Back) that are very undesirable residences, and, indeed, hardly fit for occupation.

On the whole, the accommodation for married families is extremely good, and, from a sanitary standpoint, very satisfactory.

SECTION II.

§ 1.

The following table shows the incidence of Mediterranean Fever in the different barracks and hospitals in Malta during the nine months January to September, 1905:—

Table I.

	Average population.	Number of cases.	Ratio per 1000.
Upper St. Elmo	392	26	66·38
Lower St. Elmo	613	84	137·03
Floriana	717	37	51·60
St. Francis	201	9	44·77
Manoel	474	29	61·18
Tigne	492	31	63·01
St. George's.....	1051	42	39·96
St. Andrew's (4 months)	624	15	54·00*
Verdala	618	18	29·13
Cottonera Lines	779	44	56·48
Ricasoli	639	15	23·47
Imtarfa.....	856	35	40·88
Various Barracks	650	28	43·08
Ghain Tuffieha Camp.....	473	4	8·45
Various Camps	—	14	—
Valletta Hospital	231	33	142·86
Cottonera Hospital.....	147	17	115·65
Various Hospitals	157	6	38·22
	9114	487	—

* Ratio calculated for nine months; the actual ratio for four months was 24·04.

The occupation of these barracks is as follows: Upper St. Elmo (old buildings), Tigne (principally huts and new buildings), and Ricasoli (old buildings), are occupied by Royal Garrison Artillery, of which there are eight companies (having an average strength of about 200 each), and a district establishment amounting to about another 200. Three companies are stationed at Tigne and outlying forts to the west; three companies at Ricasoli and outlying forts to the east; and two companies at Upper St. Elmo and St. James Cavalier. There has been little variation in the strength of the Royal Artillery during the year, which in January was about 2000, except a reduction during April to about 1800.

St. Francis is occupied by Royal Engineers, who have a total strength of about 380, many living in detached quarters; the numbers have not changed during the year.

Infantry battalions are accommodated in the old barracks of Lower St. Elmo, Floriana, and Verdala; in the new barracks of St. George's, St. Andrew's, and Imtarfa; in Manoel, which is partly an old fort and partly a hutment. Another battalion occupied until lately various old fortress barracks known collectively as the Cottonera Lines; and a detachment occupies Fort Chambray in Gozo. Several changes have occurred in the *personnel* of the infantry during the year. In January

the battalions present were the Sussex, Hants, Essex, West Kent, Rifles, Dublin Fusiliers, and Rifle Brigade, each from 900 to 1000 strong; and a wing of the Yorkshire Light Infantry, 500 strong. These last and the Rifles left the island in March, and the headquarters and five companies of the Sussex left in May. In February the Lancashire Fusiliers arrived, about 700 strong, being increased by about 100 in March. During April all the battalions were reduced in strength, the Essex and Rifle Brigade losing about 150 each, the West Kent and Dublins about 100 each, the Sussex and Hants about 50 each; so that during the five months, May to September, their strength was between 800 and 900, except the Hampshires, which remained at 1000.

The Departmental Corps occupy Marsamuscetto and Old Laboratory Barracks, the various hospital quarters, and hired quarters in different parts. The total combined strength has not varied, being about 350.

Valletta and Citta Vecchia Hospitals are old buildings, Cottonera and Gozo are modern, Imtarfa is a quite new building, and Forrest is an old hired house, not built for a hospital, but now many years in occupation as such.

Ghain Tuffieha is a permanent camp for Mounted Infantry, with an average strength of 180; and also accommodates battalions under training at different times during the cold season. Pembroke is a standing musketry camp, with a floating population, and Mellieha and other camps are occupied from time to time for field training.

An examination of Table I shows that the incidence of Mediterranean Fever during the first nine months of 1905 has varied considerably in different localities. A total of 487 cases in a population of 9100 gives a general ratio, for the period, of 53·52 per 1000; this varies in different places between 8·45 at Ghain Tuffieha Camp and 142·86 at Valletta Hospital. Arranged according to the severity of prevalence, the different barracks, etc., stand as follows:—

	Attack ratio per 1000.
Valletta Hospital.....	143
Lower St. Elmo	137
Cottonera Hospital	116
Upper St. Elmo	66
Tigne	63
Manoel	61
Cottonera Lines	56
St. Andrew's.....	54
Floriana	52
St. Francis	45
Various barracks and camps	43

	Attack ratio per 1000.
Imtarfa	41
St. George's	40
Various hospitals	38
Verdala	29
Ricasoli	23
Ghain Tuffieha Camp	8

The items "Various Barracks and Hospitals," and "Cottonera Lines," including many detached forts and buildings, should be disregarded in this list, the numbers occupying any individual locality being too small to enable any safe conclusion to be drawn as to factors in causation. The following points call for explanation:—(1) the excessive prevalence of the disease in the two hospitals at Valletta and Cottonera, and in the barracks at Lower St. Elmo; (2) the relative immunity of Verdala, Ricasoli, and Ghain Tuffieha; (3) the difference in prevalence between the adjoining barracks of Lower St. Elmo (137 per 1000) and Upper St. Elmo (66 per 1000), the buildings of which are within a few yards of each other.

The next table (II) shows the relative incidence in the different barracks and hospitals month by month. It is seen (1) that Mediterranean Fever, present to a slight extent in January and February, increased in March, and again very considerably in May, the increased number of cases continuing with no diminution during the rest of the hot weather; and this in spite of the fact that the strength of the troops was decreased by about 800 in April, and again by about 600 in May. (2) It is also seen that the disease did not prevail throughout the island generally with the same degree of intensity at any one time; thus at Floriana there were nine cases in March, no other barracks showing more than three or four cases in this month; after April very few cases occurred at Floriana. In May there were 11 cases in Manoel and 17 in Lower St. Elmo; but though Lower St. Elmo continued to be severely affected throughout the summer, Manoel was practically free after June. St. George's had few cases until June, and Imtarfa few until August. There was therefore a very uneven distribution of cases, month by month, which appears to contra-indicate any one general condition, climatic or other, affecting the whole barrack population.

Table II.—Distribution of Mediterranean Fever Cases, 1905.

—	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.	Total.
Upper St. Elmo	—	2	4	4	4	5	4	—	3	26
Lower St. Elmo	2	—	3	6	17	19	15	10	12	84
Floriana	2	4	9	7	2	3	4	3	3	37
St. Francis	1	3	2	—	—	1	1	—	1	9
Mancel	1	—	3	4	11	7	—	1	2	29
Tigne	—	2	4	1	7	1	6	3	7	31
St. George's	1	—	1	—	5	10	5	15	5	42
St. Andrew's	—	—	1	—	—	—	3	5	7	15
Verdala	—	2	1	1	—	5	1	4	4	18
Cottonera Lines	2	4	7	3	4	5	13	4	2	44
Ricasoli	—	1	—	—	2	3	2	7	—	15
Imtarfa	1	1	1	2	2	3	1	14	10	35
Various barracks	—	—	1	2	4	2	2	6	11	28
Ghain Tuffieha Camp	—	—	—	2	—	—	—	1	1	4
Various camps	—	—	4	4	2	—	—	2	2	14
Valletta Hospital	2	—	1	2	5	6	7	6	4	33
Cottonera Hospital	—	—	—	—	6	5	1	3	2	17
Various hospitals	—	1	—	—	—	—	2	2	1	6
Strength	12	20	41	38	71	75	67	86	77	487
	10,225	10,329	9853	9471	8661	8025	7984	7881	7855	—

Table III shows the incidence of the disease amongst the different corps stationed in Malta during the first nine months of 1905.

Table III.

	Average strength.	Number of cases.	Ratio per 1000.
Royal Garrison Artillery	1941	88	45·84
Royal Engineers.....	363	12	33·06
1st Lancashire Fusiliers (7 months)	815	51	62·58*
2nd Royal Sussex	642	31	48·29
2nd Hampshire	997	27	27·08
2nd Essex	951	84	88·33
1st Royal West Kent.....	827	37	44·78
2nd Yorkshire Light Infantry (3 months)...	539	—	—†
1st King's Royal Rifles (2 months).....	1043	2	1·92‡
1st Royal Dublin Fusiliers	900	41	45·56
1st Rifle Brigade.....	849	46	54·18
Army Service Corps	76	2	26·32
Royal Army Medical Corps	155	30	193·55
Army Ordnance Corps	80	5	62·50
Army Pay Corps.....	21	3	142·86
Miscellaneous	—	2	—
Cases occurring in hospital (not R.A.M.C.)	—	26§	—
	—	487	—

* The Lancashire Fusiliers arrived on February 27; the ratio is for the actual period of seven months; assuming the same rate of prevalence, the ratio would be 80·46 for nine months.

† The Yorkshire Light Infantry left in March.

‡ The King's Royal Rifles left in February; the ratio is for two months.

§ These are not included in the regimental figures, because removed from regimental conditions.

An examination of this table shows that the incidence varied considerably in the different corps. The general ratio throughout the troops has already been stated as 53·52 per 1000 for the period: arranged according to severity of prevalence, the corps stand as follows, only those present throughout the whole period being considered:—

	Attack ratio per 1000.
Royal Army Medical Corps	194
Army Pay Corps	143
Essex	88
Army Ordnance Corps	62
Rifle Brigade	54
Royal Sussex	48

	Attack ratio per 1000.
Royal Dublin Fusiliers	46
Royal Garrison Artillery	45
Royal West Kent.....	45
Royal Engineers	33
Hampshire	27
Army Service Corps	26

The respective numbers of the Army Service, Ordnance, and Pay Corps are so small that it would not be safe to draw any conclusions from them. The points that call for explanation are (1) the excessive prevalence of the disease amongst the R.A.M.C. and the Essex Regiment, and (2) the relative immunity of the Hampshire Regiment and the Royal Engineers.

Table IV gives the prevalence, month by month, amongst the several corps, and shows generally the same aspect of the epidemic as Table III, with which it may be compared.

§ 2.

More particular attention may now be directed to certain places where Mediterranean Fever has been especially prevalent, with a view to eliciting any circumstances that may throw light on this excessive prevalence.

Lower St. Elmo Barracks show the greatest incidence of any place, excepting the two hospitals of Valletta and Cottonera. The sanitary conditions of these barracks are shortly stated in Section I, p. 108; the water supply and drainage are in the main satisfactory, but the construction is very insanitary; the men are accommodated in case-mates, 52 feet in length, with very inadequate ventilation; the situation of the barracks, which are sunk in a hollow, is also such as to render the supply of fresh air a difficulty at all times, and practically an impossibility in calm and still weather.

From the beginning of 1905 until July 8 these barracks were occupied by the 2nd Essex Regiment; on the latter date they moved to Imtarfa, and on July 11 their place was taken by the 1st Lancashire Fusiliers. This change of occupation complicates the matter, but also to some extent helps in the investigation.

The cases of Mediterranean Fever in the Essex Regiment were distributed, month by month, as follows:—

January	2	June	19
February	0	July 1 to 8.....	7
March.....	3	July 9 to 31	9
April	6	August	14
May	18	September	6

Table IV.—Regimental Incidence of Mediterranean Fever Cases, 1905.

	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sep.	Total.
Royal Garrison Artillery	—	4	8	6	14	11	13	16	16	88
Royal Engineers	2	3	2	—	1	1	1	1	1	12
1st Lancashire Fusiliers	—	—	—	6	5	4	10	12	14	51
2nd Royal Sussex	2	4	7	2	2	4	1	4	5	31
2nd Hampshire	—	2	3	2	1	5	4	4	6	27
2nd Essex	2	—	3	6	18	19	16	14	6	84
1st Royal West Kent	1	4	8	8	2	3	4	3	4	37
2nd Yorkshire Light Infantry	—	—	—	—	—	—	—	—	—	—
1st King's Royal Rifles	1	1	—	—	—	—	—	—	—	2
1st Royal Dublin Fusiliers	1	—	1	—	4	10	5	15	5	41
1st Rifle Brigade	1	1	6	5	11	7	3	5	7	46
Army Service Corps	—	—	—	—	1	—	—	—	1	2
Royal Army Medical Corps	1	—	1	1	3	4	7	10	3	30
Army Ordnance Corps	—	—	1	1	1	—	—	—	2	5
Army Pay Corps	—	—	1	—	—	—	—	—	2	3
Miscellaneous	—	—	—	—	—	—	—	1	1	2
Hospital cases (not R.A.M.C.)...	1	1	—	1	8	7	3	1	4	26
	12	20	41	38	71	75	67	86	77	487

Of these cases all, up to July 8, were admitted from Lower St. Elmo; with the exception of two in March, admitted from Pembroke Camp; and three in May, one in June, and one in July, which were admitted from Gozo. From July 9 onwards all the cases were admitted from Imtarfa Barracks, except one from Gozo and one from Ghain Tuffieha. It has been already stated that it is necessary to allow a period of 14 days for incubation in most instances, and that probably a *further period* of 14 days should be allowed in many instances, between date of contracting infection and date of admission to hospital; cases admitted more than 28 days after departure from any particular place can hardly be considered to be due to infection originating in that place. If this limit be provisionally adopted, the two cases admitted from Pembroke Camp should be referred to Lower St. Elmo, as also should three of the cases admitted from Gozo. *Per contra*, one case admitted from St. Elmo in May, 13 days after arrival from Gozo, may be debited to the latter place, though, in this instance, some doubt must be felt as to the length of the incubation period.

The 2nd Essex Regiment arrived in Malta from England, and took up quarters in Lower St. Elmo, on April 29, 1904; here six companies remained throughout the year, except for short periods at Pembroke, Mellieha, and Ghain Tuffieha Camps, returning from the last-named on December 19, 1904. Two companies were stationed at Gozo (A and B) from April 29, to September 1, 1904, on which date they were relieved by D and F Companies, who remained there until May 8, 1905; being relieved in turn, on that date, by C and E Companies.

Of the total number of cases (55) occurring in the regiment between January 1 and July 8, 1905, all, except one, belonged to the six companies stationed at Lower St. Elmo; only this one came from the two companies stationed at Gozo.

The incidence of Mediterranean Fever in the different companies during the whole period of nine months, January to September, was as follows:—

A.	B.	C.	D.	E.	F.	G.	H.	Uncertain.	Total.
11	15	4	2	3	8	19	19	3	84

The companies that have been stationed throughout the whole time at Lower St. Elmo, and subsequently at Imtarfa, have been A, B, G, H; these four companies have had 64 cases; the four companies, of approximately the same strength, that have been part of the time at Gozo (C, D, E, F), have had 17 cases; of these 17, four were admitted from, and without doubt contracted the fever in, Lower St. Elmo; seven probably contracted the disease there, and four probably at Imtarfa; in two instances only, or perhaps three, was it contracted at Gozo.

It is necessary to distinguish between what is practically certain, and what is uncertain. Assuming the correctness of the diagnosis, as to which every care has been taken (as mentioned in p. 108), there is little doubt that 84 cases of Mediterranean Fever have occurred in this battalion. As to the place where infection was contracted, there is no such certainty, because the incubation period has not yet been definitely ascertained. It is a fact to be noted that 64 of these 84 cases occurred in the four companies A, B, G, H, which remained the whole time in Lower St. Elmo and Imtarfa; in three instances, two of which were from the mounted infantry at Ghain Tuffieha, the company to which the man belonged is not ascertained: of the 17 cases occurring amongst the other four companies, 11 probably originated in Lower St. Elmo. There is therefore a presumption that the excessive prevalence of the fever in this battalion was due to something belonging to, or connected with, Lower St. Elmo Barracks.

These barracks consist of a range of building in three stories, the rooms in the lowest being used as stores, cookhouse, canteen, etc., and the middle and upper stories being used as barrack rooms. They were allocated thus:—

Upper Storey—

Nos. 1 to 3.—C Company, January 1 to May 6.

D Company, May 8 to July 8.

4.—Signallers.

5 to 8.—A Company all the time.

9 to 12.—E Company, January 1 to May 6.

F Company, May 8 to July 8.

13, 14.—Drums.

Middle Storey—

Nos. 1 to 4.—B Company

5 to 8.—G Company

9 to 12.—H Company

13, 14.—Band

} all the time.

The actual barrack rooms from which cases were admitted are not always ascertainable with accuracy; the following particulars as to the rooms occupied are as much as I have been able to find out:—

Upper Storey.

Nos. 1 to 4.—Cases were admitted May 9, 31; June 2 (C Co.); July 31 (D Co.); total, 4 cases.

Nos. 5 to 8.—Cases admitted March 18; May 20, 21, 31; June 6, 7; July 1, 5, 11; August 4; total 10 cases (A Co.); one other case in this company lived at the sergeants' mess.

Nos. 9 to 12.—Cases admitted April 12, 22; May 1 (E Co.); July 13, 24, 28 (F Co.); total, six cases.

Nos. 13, 14.—Cases admitted May 23 (H Co.); June 10; July 2 (G Co.); total, three cases.

Middle Storey.

Nos. 1 to 4.—Cases admitted January 22; March 16; April 6; May 2, 8, 19, 31; June 2, 10, 21; July 20; total, 11 cases in B Co., of which four at least were in No. 2, and three at least in No. 3 room.

Nos. 5 to 8.—Cases admitted January 22; April 1; May 3, 26; June 8, 10, 11, 16, 21, 26, 30; July 24, 31; total, 13 cases, all in G Co., of which four at least lived in No. 8 room; two other cases in this company were drummers, living in Nos. 12 or 13, Upper Storey.

Nos. 9 to 12.—Cases admitted March 25; April 5, 18; May 3, 20, 27; June 5, 11, 17, 28; July 2, 4, 20; August 4 (two cases); total, 15 cases, all in H Co.; of these at least three were in No. 10, and three in No. 12. One other case in this company occurred in the sergeants' rooms on the Upper Storey.

Nos. 13, 14.—One case occurred, May 6; Band, F Co.

The above list accounts for 65 out of the 84 cases that occurred in the battalion. After they moved to Imtarfa on July 8, cases continued to occur, and of these 12 have been regarded as probably due to St. Elmo infection, up to August 5. After this date infection has been considered to have been contracted at Imtarfa, though very possibly introduced from St. Elmo. Three cases were admitted from G Company on August 6 and 7, which might be thought to be more likely due to a continuance of the same influence, whatever it was, that caused the special incidence on this company, with an extra long period of incubation. Eight other cases occurred up to the end of the month, and six in September, there being a very notable diminution in this month.

Before the arrival of the Essex Regiment at Imtarfa, a few cases had been admitted from the Sussex Regiment, which had been quartered in these barracks since February 22. Besides five cases in the middle of March, probably dating from Polverista, there had been at Imtarfa one case in March, two in April, two in May, two in June, and one on July 5; the disease was present, but did not prevail at the station; the average strength of the troops during January to June was 880. No cases occurred in the Sussex Regiment after July 5 until August, in which month there were four admissions from this battalion. Granting that the length of the incubation period

is uncertain, if one compares the considerable prevalence of the fever at Lower St. Elmo with its trivial manifestations at Imtarfa during the early part of the summer, it appears more probable that the Essex cases occurring at Imtarfa were due to a "something" brought up with the regiment from St. Elmo than to any infection of local origin at Imtarfa. Whether this supposed infective "something" was brought up as an already ingested but latent *contagium*, within the bodies of the men who afterwards developed the disease, or whether it was introduced in fomites, or infective matters external to the body, is a question to be considered further. At this stage we are, I think, to some extent justified in the presumption that the Imtarfa cases, for at any rate four weeks and possibly longer after arrival at the new quarters, were due not to anything belonging to Imtarfa, but to some factor that had been in operation at St. Elmo, and which continued in operation for some time afterwards. The drop from 14 admissions in August to six in September is noteworthy.

It is worth while considering a converse instance; D and F Companies went from St. Elmo to Gozo on September 1, 1904, and returned to St. Elmo on May 8, 1905. From these two companies two cases of Mediterranean Fever were admitted on September 8, 1904, one on September 18 and one on October 3; the first three and perhaps the fourth case may be presumed to have become infected before leaving St. Elmo; a fifth case was admitted on October 8 (38 days after leaving St. Elmo), and not a single other case occurred during their eight months' stay at Gozo, nor (with one exception) during their stay at St. Elmo between May 8 and July 8; four cases were admitted at Imtarfa during the latter half of July. The one case admitted after return to St. Elmo that appears to have contracted the infection in Gozo (Private Lawrence, F Company) was admitted on May 21, 13 days after arrival. Whatever the conditions were that led to the prevalence amongst the occupants of Lower St. Elmo, they appear to have been absent from Gozo even more completely than from Imtarfa. The immunity enjoyed by these two companies while at Gozo continued for two months after their return to St. Elmo, yet in G and H Companies 21 cases occurred in the same two months, May 8 to July 8, 1905.

The 2nd Essex having marched up to Imtarfa on July 8, their quarters in Lower St. Elmo were taken over by the 1st Lancashire Fusiliers, who marched in on July 11. This regiment had landed in Malta from England on February 27, 1905, and on arrival were quartered in Polverista Barracks; they marched to Pembroke Camp on March 20, thence to Mellieha Camp on April 30, and returned to Polverista on May 8, remaining there until their move to Lower St. Elmo. Six companies then occupied these barracks, and two (B and D) went into Manoel Hutments.

The first admission to hospital for Mediterranean Fever occurred on April 5, that is 37 days after landing in Malta; the second admission was on April 17; until their move to St. Elmo there were 18 other admissions, that is 20 in 15 weeks; during the same period the Essex Regiment had 50 admissions; the prevalence in the Lancashires was, therefore, much less than in the Essex. During the next four weeks eight cases were admitted from Lower St. Elmo, having presumably contracted infection in Polverista. From August 9 to the end of September, 19 cases occurred in the six companies in St. Elmo, and three cases in the two companies occupying Manoel, presumably due to infection contracted in those places.

In this regiment, unlike the Essex, the incidence on different companies has varied but slightly. During the whole period from their landing until the end of September (seven months) the distribution of cases has been as follows:—

A.	B.	C.	D.	E.	F.	G.	H.	Total.
5	4	9	8	8	5	4	8	51

There is here no marked preponderance, as is the case of B, G, and H Companies of the Essex. Before the move to St. Elmo the distribution had been thus:—

A.	B.	C.	D.	E.	F.	G.	H.	Total.
2	1	2	7	2	4	1	1	20

Within the next four weeks the cases occurred thus:—

A.	B.	C.	D.	E.	F.	G.	H.	Total.
1	1	2	0	2	0	1	1	8

From August 8 until September 30 the cases occurred thus:—

A.	B.	C.	D.	E.	F.	G.	H.	Total.
2	2	5	1	4	1	2	6	23

In the first set of 20 cases D Company certainly furnishes a disproportionate number, but no common origin is apparent, and the cases were spread over three months. It may be noted that two cases in A company were admitted from the same room (No. 3 Polverista) and two cases in F Company also from one room (No. 14 Polverista). These rooms accommodate 14 men in each. The battalion was quartered in a number of small barracks, including not only Polverista itself, but also all the others making up Cottonera Lines, and was therefore widely scattered. On coming to Lower St. Elmo six companies were concentrated in one building under like conditions. It is to be noted that seven cases occurred in C Company, which occupied Nos. 5 to 8 rooms in the middle storey, the same that had just been vacated by G Company of the Essex Regiment; this company had furnished 19 cases of Mediterranean Fever, 13 of which

appear to have contracted infection while occupying these four rooms, seven of these having been admitted within the preceding three weeks. Of the seven cases in the Lancashires one was a colour-sergeant who occupied the same room as a colour-sergeant of the Essex, admitted with Mediterranean Fever eight weeks before; of the other six, three cases came from No. 7 room, a room from which two cases at least had been admitted in the Essex Regiment within the preceding six weeks. Is it a mere coincidence that six men should be admitted from the same four rooms as had furnished seven cases in another regiment during the few weeks immediately preceding?

The other company that showed a relatively large number of admissions after arriving at St. Elmo was H Company; seven cases occurred between July 11 and the end of September; they were admitted from the four rooms, Nos. 10 to 13 in the upper storey; two of these came from the same room, No. 12, on September 22 and 25 respectively. These rooms had been occupied between May 8 and July 8 by F Company of the Essex Regiment, which had arrived from Gozo on May 8, and did not show any especial prevalence during its stay at St. Elmo; shortly after arriving at Imtarfa, however, three cases occurred, on July 13, 24, and 28, which had presumably contracted infection when staying in the rooms now mentioned.

With regard to the other group of cases, in C Company, the position may be stated thus: We have a body of men that have been living in Malta for rather over four months, and in that period have furnished two cases of Mediterranean Fever, apparently arising at two different places (Pembroke Camp and Corradino Prison), and with a month's interval between the two, the last admission having been six weeks since. This body of men now move into fresh quarters consisting of four rooms, just vacated by another group of persons, who have furnished 11 cases of fever during the year up to the date of leaving, seven having been admitted during the preceding three weeks. Out of the new company four are seized with the fever within six weeks. There is, I submit, a presumption that the infective agent is connected with the *place*. If this be considered a reasonable presumption, a further point to be noted is that the first admission amongst the fresh body of men occurred 20 days, and the second 22 days, after their arrival at the presumably infected barrack rooms, indicating an incubation period of about 21 days.

An examination of the incidence of Mediterranean Fever in these two bodies of men, the Essex and the Lancashires, so far indicates that the disease may prevail in a strictly localised fashion. Both these battalions have occupied old barracks with many sanitary defects; the next case to be considered is that of a battalion living in good modern barracks in a healthy situation.

§ 3.

The First Royal Dublin Fusiliers have occupied St. George's Barracks, Pembroke, since March, 1904; they arrived in Malta from South Africa in November, 1902; in February, 1903, five companies left for Crete and Cyprus, the remaining three being stationed at Imtarfa. At the beginning of March, 1904, the whole battalion came into St. George's. During 1904, only four cases of Mediterranean Fever occurred, one of which was contracted in Cyprus. Therefore, the battalion and the barracks were almost exempt from the disease during 1904. During the nine months, January to September, 1905, there have been 41 admissions. The different companies have suffered very unevenly, thus:—

A.	B.	C.	D.	E.	F.	G.	H.	Total.
13	6	5	3	8	2	3	1	41

It is seen that A Company has suffered most, with 13 cases; and H Company least, with only 1 case. In A Company there was a sequence of 5 cases between June 16 and July 6; and again a sequence of cases between August 16 and September 15; in D Company 3 cases occurred close together between August 16 and 30; and in E Company there were 3 cases between August 11 and 14, and again 3 cases between August 27 and September 10.

There is a noteworthy circumstance in connexion with the company incidence of Mediterranean Fever in this regiment: A Company, which had 13 cases, and H Company, which had only 1 case, occupy the same barrack block, lettered F; the companies are of the same strength; they each occupy seven barrack rooms; A Company having those in the eastern half, nearest the sea, and H having those in the western half. The two companies use the same cookhouse, the same ablution room, the same latrine and urinal. The barrack block is one of six single-storey buildings, and consists of 14 rooms, all alike, measuring 30 × 21 feet by 14 feet in height, and accommodating 13 men in each, with an allowance of 605 cubic feet per head. Although there are no openings in the sides of the rooms, the door and two windows at each end, and the roof ventilators, appear to provide sufficient means of entrance and exit for the free circulation of air; the rooms seem to be airy and well ventilated. There are also in each block six bunks for non-commissioned officers; these are placed back to back, with no through ventilation, and are certainly hot and stuffy; none of the fever cases in this company occupied any of these bunks; the sergeant, who was admitted on May 29, lived in A Block, Married Quarters. Though these barracks are not of the most modern type, I can find no fault

with their essential sanitary conditions ; the cookhouses and ablution room are excellent, the drainage system is modern, and, with trifling exceptions, of good construction ; the drains are kept in excellent order, and the sanitation of the lines carefully attended to. The one important sanitary defect is the scarcity of water for flushing purposes, leading to a foul state of the water latrines of occasional, or perhaps frequent, occurrence. As regards situation, nothing better could be desired ; they form a complete contrast to Lower St. Elmo and Floriana Barracks, and the extremely objectionable places occupied by troops in Cottonera Lines. They are freely exposed to the air on all sides, with no habitations near, and in three directions are practically open to the sea. Complaint was made by some men occupying No. 14 room (the one nearest to the sea) of bad smells coming from the sewer outlet into the sea in this direction. With the existing scarcity of water for flushing purposes, no doubt the drain air is occasionally offensive and the sewage malodorous ; but I do not think that any harm could result to the occupants of these barracks therefrom ; the outlet is many hundred yards distant from any of the buildings.

There does not appear to be any condition affecting A Company that does not equally affect H Company, and as the latter has been immune to Mediterranean Fever throughout 1904 and 1905, to the end of September (with the exception of one case), the cause for the prevalence in A Company is not obvious. The drinking-water supply and the milk supply are common to the whole regiment ; the milk has been boiled, not by companies separately, but by the master cook centrally for all ; therefore, whether effectually sterilised or not it has been consumed in the same—boiled or unboiled—condition by all companies alike.

In these barracks, as in most of the older barracks in the island, it has been the practice to affix the accoutrement shelves to the walls of the rooms in a continuous line, and not to separate them from each other as far as the linear space will allow. It is the custom to arrange the bed-cots in symmetrical order, each under the shelf that holds the occupant's kit and accoutrements. The length of the shelf is 3 feet 5 inches, the width of the bed is 2 feet 5 inches ; consequently, when the shelves and beds are so arranged, there is only a space of 12 inches between bed and bed. Although the occupants have each 605 cubic feet of air space in the room, they are not evenly distributed so as to get each one his fair share, his one-thirteenth part, of the total cubic space ; but they are crowded together, with only a foot between each pair of beds. Now, whatever the *materies morbi* may be, if it be once introduced into a room arranged like this, infection from person to person will the more readily take place, the closer the men are crowded together. So that if we assume that there is a particulate *contagium*, capable of being conveyed from an infected to a non-infected

person, such an arrangement as now described would facilitate spread of the disease, when the *contagium* has been once introduced, or has been introduced in sufficient quantity. But until it has been introduced, the arrangement would have no effect. In the case of these barracks, in which the sanitary conditions are uniform and (except for aggregation of beds and scarcity of flushing water) satisfactory, this aggregation offers a plausible explanation of the *spread* of the fever, though not of its origin.

§ 4.

There are eight companies of Royal Garrison Artillery quartered in Malta, their total average strength for the first nine months of 1905 amounting, with the District Staff, to 1941: in this body of troops there occurred 88 cases of Mediterranean Fever (besides seven cases contracted in hospital), giving a ratio of 45·34 per 1000. There was, however, considerable variation in the prevalence in the different companies, as shown in the following table:—

Royal Garrison Artillery.

	Strength.	Cases.	Attack ratio per 1000.
No. 1 Company.....	190	13	68·4
5 " "	235	8	34·0
63 " "	216	4	18·5
65 " "	205	17	82·9
96 " "	217	15	69·1
99 " "	222	12	54·0
100 " "	240	10	41·7
102 " "	215	7	32·5
District Staff	196	2	10·2

The men of the Garrison Artillery in Malta are, as a rule, older and of greater length of service, than the average infantry soldier: a larger proportion also have already had foreign service. As to length of stay in the island, two of these companies (63 and 99) arrived in 1902, two (99 and 100) in 1903, and the other four in 1904; it is not unusual, however, for men to exchange from one company to another, and under present arrangements the companies do not move from station to station *as a body*, but change their *personnel* by individual reliefs. The two companies that have been longest in Malta show a great difference in their fever incidence, No. 63 having had 18·5 cases per 1000, and No. 96 having had 69·1 cases per 1000; Nos. 99 and 100 arrived together, and show a similar incidence, 49·5 and 41·7 per 1000; Nos. 1, 5 and 65 arrived together, but show an incidence very unequal, viz., 68·4, 34·0 and 82·9 per 1000: No. 102, which arrived three months earlier, only 32·5 per 1000. It does not appear that

length of residence in Malta can account for the great variation in this year's prevalence of the fever in the Artillery.

The variation, however, has a relation to the barracks in which the different companies have been quartered. Two companies have been stationed at St. Elmo, three companies at Tigne (with a detachment at St. George's for a part of the time), and three companies at Ricasoli ; thus :—

Upper St. Elmo—

No. 65	17 cases	82·9 per 1000
96.....	15 „	69·1 „

Tigne—

No. 1.....	13 „	68·4 „
99.....	12 „	54·0 „
102.....	7 „	32·5 „

Ricasoli—

No. 5.....	8 „	34·0 „
63.....	4 „	18·5 „
100.....	10 „	41·7 „

St. James Cavalier, etc.—

District Staff	2 „	10·2 „
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A portion of 65th Company has been accommodated in St. James Cavalier and Castille, but only one case of fever has occurred among the men of this detachment. All companies have from time to time sent detachments to the outlying forts and practice camps ; two cases have occurred in such detachments from 65th Company, and one from 96th Company. If these be deducted from the St. Elmo figures the incidence on the troops stationed there will be lessened, but the disproportion between the different barracks will not be diminished ; for out of the 10 cases in 100th Company, no less than five were apparently infected at outlying forts ; and of the seven cases in 102nd Company two were similarly of outside origin. The numbers are too small to justify any important deduction, but it is certainly noteworthy that the companies quartered in Valletta have been much more severely affected than those at Ricasoli, those at Tigne occupying an intermediate place.

The barracks at Upper St. Elmo are not quite satisfactory, but are not nearly so bad as Lower St. Elmo, or St. James Cavalier ; the chief points are—defective latrine and urinal arrangements, and the continual use of tents, which must lead to fouling of the ground. St. James Cavalier is a very bad barrack, overcrowded and impossible to ventilate, with insanitary latrine conditions owing to scarcity of water : Mediterranean Fever has not however been prevalent there. On the other hand the sanitary condition of Fort Ricasoli is, on the whole, satisfactory, except that the large barrack rooms are difficult to

ventilate: the situation of this fort is ideal; as is that of Tigne barracks, which last are in as good a state, sanitarily, as Ricasoli: the incidence of Mediterranean Fever has however been greater at Tigne. I am not able to trace any definite connexion between the degree of prevalence of Mediterranean Fever amongst the different artillery units and the respective sanitary conditions of the barracks in which the units were quartered.

The isolation of Ricasoli from all native surroundings is fairly complete: this may be said in a lesser degree of Tigne: but from Tigne the men habitually go to spend their spare time in Valletta, whereas from Ricasoli it is not, I am informed, at all common for the men to cross the Grand Harbour to spend their evenings in the town. Isolation (habitual though not complete) does not however prevent the occurrence of the disease, as 11 cases have occurred amongst men stationed in outlying forts and camps, the disease having apparently been contracted in these places. Though situated in the open country, and generally near the sea, so as to be airy and healthy, there are certain insanitary conditions present in nearly all these forts. From the necessity of the case the sleeping rooms are imperfectly ventilated, though I did not find that there had been any overcrowding anywhere, except at Benghisa. In most forts the water supply was scanty, leading to parsimony in its use for latrine and urinal flushing, where water latrines are provided. Dry earth latrines are, however, in general use; and, with some few exceptions, are kept in an insanitary condition. With a scanty water supply, a very imperfect method of removal of excreta, and a minimum amount of air space, or air change, in the sleeping rooms, the forts cannot be called sanitarily satisfactory; but as regards the general health of the men quartered in them, it appears to be excellent, in spite of these sanitary shortcomings. Two cases were admitted from Fort Delimara on the same day (August 10), and two cases from Ta Silch within a week of each other (September 6 and 13); in no other instance was there any apparent connexion between cases at any of the forts.

Three cases were admitted on three successive days, May 18, 19, 20, in No. 65 Company, from tents at Upper St. Elmo, but I was unable to ascertain if they had occupied the same tent. Two cases occurred out of eight occupants of the same room, No. 6 Lower Storey, in 96th Company, on March 23 and April 19. Two cases occurred in 99th Company in the same hut, No. 6; and two other cases also in the same hut, No. 8, at Tigne, within a fortnight of each other in September; there were 16 occupants in each hut. Within the space of 14 days in August three men were admitted from No. 1 barrack room, Ricasoli, in 5th Company, accommodating 33 men; and two men, out of five occupying a room at the Auberge de Castille, and employed at the Royal Artillery officers' mess, were admitted on

June 9 and July 29. What connexion there may have been between these cases is doubtful. No. 1 room at Ricasoli is especially difficult to ventilate; the room at the officers' mess is reported to be close and stuffy; the Upper St. Elmo rooms and the Tigne huts are sanitarily satisfactory.

§ 5.

There are two infantry battalions that have not suffered to any great extent from Mediterranean Fever during 1905, but which offer a contrast, the one to the other. The 1st Royal West Kent Regiment arrived in Malta on April 15, 1904, and after a month in camp at Pembroke and Mellieha were quartered in Floriana Barracks, where they have remained ever since, except for a month in camp in November and December, 1904, and again three weeks in March, 1905. One company however, B, was in camp at Ghain Tuffieha for three months from February 1 to May 1. During the nine months 37 cases of Mediterranean Fever were admitted from the battalion, the distribution by companies being as follows:—

A.	B.	C.	D.	E.	F.	G.	H.	Uncertain.	Total.
6	1	4	1	5	3	7	8	2	37

The barracks occupied by this regiment are of a composite character. Three companies have been quartered in the "Old Barracks," which are casemates of the type so common in Malta; these are especially difficult to ventilate, on account of their great length, which is about 80 feet; they have however not been overcrowded, as the battalion has not been up to full strength, and the cubic space has therefore been nominally sufficient; instead of 30 men, not more than 24 have generally occupied one room. It is however evident that an apartment 80 feet in length, with no lateral openings whatever, cannot possibly be supplied with a proper change of air by natural ventilation. Three companies have been quartered in the New Barracks, which consist of three separate two-storey blocks, constructed with every regard to the principles of modern sanitation; each room accommodates 26 men. One company has been quartered in Notre Dame Ravelin Barracks, a single storey building consisting of 16 small rooms, with five men in each; and one company has been divided between one room in the Old Barracks and two huts in the Ravelin; the band also occupy huts in the Ravelin; each hut accommodates 18 men. A small detached fortress, the Salvatore counterguard, accommodates a party of signallers, numbering 30.

The number accommodated in the Old Barracks is 360, in the New Blocks 318, in the Ravelin and Salvatore 245; total, 923. The distribution of cases of Mediterranean Fever has been thus:—

A, C, and G (Old Barracks)	17 cases.
B, E, and F (New Barracks)	9 „
D (Ravelin)	1 „
H (Old Barracks and Ravelin)	8 „
Uncertain.....	2 „
	<hr/>
	37 „

Three companies occupying the Old Barracks have had 17 cases; three occupying the New have had nine cases; one company occupying the Ravelin has had one case, and H Company, divided between the Old Barracks and Ravelin, has had eight cases. One case, however, in A Company was admitted, not from Old Barracks but from Notre Dame Ravelin (a bandsman); the one case in B Company, and one case in E, occupied huts in Ravelin, not the New Blocks; and one case in H was infected apparently in Pembroke Camp, not in the Old Barracks. Making these corrections it is seen that the admissions were as follows :—

From Old Barracks, accommodating 360.....	23
From New Barracks, accommodating 318	7
From N.D. Ravelin, &c., accommodating 245...	4
From Pembroke Camp (1), Ghain Tuffieha (1)	2
Uncertain	1
	<hr/>
	37

It seems evident that the incidence on the body of men occupying the Old Barracks is disproportionate, although the numbers are too small to afford much ground for drawing any decided conclusion. In two instances there seems to have been a likely connexion between cases; in A Company two admissions took place from No. 8 room, one on March 28, the other on April 10; in H Company three men were admitted from No. 3 room, on April 2, 9, and 24; the exact room that a man slept in is not ascertainable in every instance, although it is generally possible to localise the part of the barracks occupied with some accuracy. It is quite clear that the battalion, as a whole, did not suffer uniformly, but that there was a prevalence amongst some groups of men, or in some portions of the barracks.

§ 6.

The 2nd Hampshire Regiment has furnished fewer cases of Mediterranean Fever during the period under consideration than any other regimental group: there have been 27 cases in a strength of 997 men. They arrived in Malta on September 16, 1903, and have remained in

Verdala Barracks all the time, with short yearly absences in camp. A portion of the battalion has occupied some of the smaller barracks in Cottonera Lines (Polverista, St. Clement's, etc.), but no especial difference is observable in the incidence of the fever upon these different detachments, the ratio for the whole battalion being 27 per 1000, and for the Verdala portion 29·1 per 1000. The cases occurred sparsely throughout the year, not more than six occurring in any one month (September), and no company contributing more than four; in only one instance has there appeared to be any connexion between one case and another: a man was admitted from Zabbar Gate on July 6, and another from the same place on July 15.

The general sanitary condition and surroundings of Verdala and the Cottonera Lines are certainly not better than those of Floriana, and in one important respect, that of scanty water supply for flushing purposes, they have been much worse off; the small casemate rooms in Verdala are, however, much better ventilated than the large casemates in Old Floriana Barracks.

The 2nd Royal Sussex Regiment arrived in Malta from England on June 27, 1904, and were quartered in Polverista and other barracks in the Cottonera Lines. They were in camp at Pembroke and Mellieha, four companies at a time, for a month in November and December; and the whole battalion together at Ghain Tuffieha for a fortnight in January, 1905. They marched up to Imtarfa on February 22 and 26, and on May 29 five companies, with the headquarters, left for Crete. During 1904 they suffered little from Mediterranean Fever, having had only 11 admissions altogether; up to the departure of the five companies to Crete at the end of May, there had been 17 admissions during 1905; these cases had been scattered about in different companies and barracks, and there was no particular incidence on any one company or barrack. Between June 26 and August 24 there were five admissions from C Company, all from the same barrack block (H), and two from the same room (No. 97), on August 4 and 10; from this room also another man was admitted on September 21. H Company occupies the other half of H block, from which four other cases were admitted between April and September, but no two from the same room. The rooms vary in size, some accommodating 16, and some 20 men; No. 97 accommodates 20 men.

§ 7.—*Hospitals.*

The total number of cases of Mediterranean Fever that apparently contracted the disease in hospital, either as patients admitted for some other illness and subsequently developing this fever under circumstances pointing to hospital infection, or as non-commissioned officers and men of the Royal Army Medical Corps, and other men attached

for nursing duties, amounted to 56. Of this number 33 occurred at Valletta Hospital, 17 at Cottonera. The accompanying table shows the distribution in detail. It is seen that, in all, 23 patients in hospital contracted the disease, 30 R.A.M.C., and three men attached for nursing duties :—

—	Average number of patients.	Average number R.A.M.C.	Cases of Mediterranean Fever.		
			Patients.	R.A.M.C.	Men attached.
Valletta	157	74	11	19	3
Cottonera	102	45	10	7	—
Forrest	47	10	1	2	—
Citta Vecchia...	47	15	1	2	—
Imtarfa	25	8	—	—	—
Gozo	2	3	—	—	—
	380	155	23	30	3

The total average hospital population, including patients and orderlies, amounted to 535, amongst whom there occurred 56 cases of Mediterranean Fever, being in a ratio of 104·66 per 1000. Of these, 23 occurred in an average population of 380 patients, being in a ratio of 60·53 per 1000 ; and 30 in an average population of 155 orderlies R.A.M.C., or, 193·55 per 1000. While the incidence is decidedly greater on the men of the R.A.M.C., this varies in the different hospitals ; thus at Valletta the ratio among the patients was 70·1, among the R.A.M.C. 256·8 per 1000 ; at Cottonera, among the patients 98·0, among the R.A.M.C. 155·6 per 1000. The incidence upon the patients is not, however, fairly comparable either with that upon the R.A.M.C., or with that upon any of the regimental units that have been previously considered ; because the hospital *sick population* is constantly changing. The R.A.M.C. prevalence can be fairly compared with that of any other unit, and the severity of the outbreak amongst this body of men is immediately evident. The highest ratio in any regimental unit is that of the Essex Regiment, 88·33 per 1000 : and for any barracks, that of Lower St. Elmo, 137·03 per 1000 ; for the R.A.M.C. as a whole the ratio is 193·5, for those quartered in Valletta Hospital 256·8, for those at Cottonera Hospital 155·6 per 1000.

The incidence upon the patients at Valletta Hospital, 70·1 per 1000 was less than that upon the Essex Regiment ; at Cottonera the patients suffered more, viz., 98·0 per 1000 ; but these ratios are not properly comparable, as just mentioned.

The circumstances that lead to the opinion that the infection was contracted in hospital in the following instances will now be shortly stated, beginning with the sick under treatment for other forms of illness :—

Case 1.—Private Minter, Essex Regiment, was admitted from Lower St. Elmo to Valletta Hospital on February 14, with "gonorrhœa"; on March 25 he was transferred to Forrest Hospital; his "disease" was changed to Mediterranean Fever on April 20; it is almost certain that infection was contracted in one or the other hospital, more probably in Valletta than in Forrest.

Case 2.—Private Salmon, Rifle Brigade, was admitted to Valletta Hospital for "debility" from Manoel Hutments, on March 24, 1905. He was treated in 20 A Ward. He was transferred to Citta Vecchia Sanatorium, April 17; and discharged to duty at Manoel, May 1. He was again admitted to Valletta on May 14, having been ill for about 10 days. His illness commenced in the first week of May, and was contracted either in Valletta or Citta Vecchia Hospitals, most probably in the former.

Case 3.—Gunner Jardine, R.G.A., was admitted to Valletta Hospital from Upper St. Elmo suffering from "orchitis" on April 3; he was transferred to Citta Vecchia on May 22, and the "disease" changed to Mediterranean Fever on June 1; his infection was almost undoubtedly contracted in Valletta Hospital.

Case 4.—Gunner Moore, R.G.A., was admitted to Valletta Hospital from Upper St. Elmo with "gonorrhœa" on April 7; his "disease" was changed to Mediterranean Fever on June 13, after more than two months' stay in hospital, during which time he must have taken the infection.

Case 5.—Private Bush, Essex Regiment, was admitted from Lower St. Elmo to Valletta, suffering from venereal disease, on May 24; on June 6 he was transferred to Cottonera, and on June 26 began to be ill with Mediterranean Fever; the infection was probably contracted within the preceding 33 days, i.e., after his admission to Valletta Hospital; but it is uncertain whether at Valletta or Cottonera; moreover, Mediterranean Fever was prevalent at Lower St. Elmo in May. This is a doubtful case of hospital infection.

Case 6.—Private Potter, Rifle Brigade, was in Valletta Hospital with venereal disease from May 16 to June 7, when he was discharged to St. Andrew's Barracks. He was admitted to Forrest Hospital on June 29, suffering from Mediterranean Fever, having been ill for about one week before this; it is more probable that he took infection in Valletta Hospital before June 7, than in St. Andrew's Barracks between June 7 and 22; these barracks had just been completed and taken into occupation on June 2.

Case 7.—Private Gerard, Rifle Brigade, was admitted to Valletta from Ghain Tuffieha Camp on May 14, suffering from gonorrhœa; on July 1 Mediterranean Fever was diagnosed; infection was almost certainly contracted during the preceding six weeks in hospital.

Case 8.—Private Wilding, Rifle Brigade, was admitted to Valletta, 20 A Ward, from Manoel on May 30, with enteric fever; on July 16 he was found to be suffering from Mediterranean Fever and the "disease" was changed; infection was probably contracted during the preceding 46 days in hospital; unless it be supposed that a double infection had been contracted originally, and that the enteric symptoms and agglutination phenomena had masked those of Mediterranean Fever.

Case 9.—Gunner Marjerum, R.G.A., was admitted to Valletta on July 29, with Mediterranean Fever, having only been discharged from the same hospital 10 days before, during three or four of which he was sickening with the fever; he

had previously been under treatment in 20 B for six weeks (gonorrhœa), and in this period probably contracted the infection.

Case 10.—Private Heaton, Lancashire Fusiliers, was admitted to Valletta from Lower St. Elmo on August 1, with Mediterranean Fever, having already been ill a few days. From June 28 to July 15 he had been in the same hospital, treated for "debility," but without any symptoms of Mediterranean Fever. It is more probable that infection was taken during the fortnight before than during the fortnight after, July 15; but the case is an uncertain one. The battalion had moved from Cottonera Lines to Lower St. Elmo on July 11; several cases of fever had occurred, but not in this man's company, about that time.

Case 11.—Private Keylock, Hants Regiment, was admitted to Valletta Hospital with gonorrhœa on July 29; he was discharged on September 1, and readmitted, suffering from Mediterranean Fever, on September 15. It is almost certain that he contracted the infection during his stay in hospital.

Case 12.—Private Collins, Hants Regiment, admitted to Cottonera March 23, 1905, suffering from enteric fever, as shown by serum reaction; serum reaction was negative to Mediterranean Fever, March 27 and April 29, but positive on May 1, when he had been in hospital 38 days; infection was therefore probably contracted in hospital, though conceivably along with the enteric infection before admission.

Case 13.—Private Bishop, Hants Regiment, admitted from Verdala to Cottonera, March 30, with scarlet fever; was isolated in No. 12 Ward from this date until April 26, when he was transferred to No. 5 Ward, where he remained until discharge on May 2; was readmitted May 10, and diagnosis made of Mediterranean Fever on May 15. His illness came on suddenly on May 9, and was almost certainly contracted between March 30 and May 2, while he was in Cottonera Hospital.

Case 14.—Gunner Abbott, 65th Company, Royal Garrison Artillery, was admitted from Upper St. Elmo to Cottonera, May 16, and placed under observation for mental disease. After being in hospital under close observation for 30 days symptoms of Mediterranean Fever developed, and the diagnosis was made on June 21. The length of sojourn in hospital before onset of fever symptoms points to infection contracted within the hospital precincts. It is to be noted, however, that three men in 65th Company were admitted with Mediterranean Fever on May 18, 19, and 20 (having presumably been ill a few days before, and conceivably infectious); and that other admissions from the same company for this disease took place on June 1, 3, 16, 19, and 20; infection was therefore present in this company in Upper St. Elmo.

Case 15.—Private Haines, Hants Regiment, was admitted from Verdala to Cottonera, No. 10 Ward, with "abscess," on April 21; Mediterranean Fever was diagnosed May 19; there were fever cases in No. 10 Ward at the time of his admission; it is more probable that infection was taken during the 29 days' sojourn in this ward than in Verdala Barracks previous to admission; in these barracks one case only had occurred in March, one in April, and none in May.

Case 16.—Gunner Duncan, 63rd Company, R.G.A., was admitted from Ricasoli to Cottonera, No. 1 Ward, with gonorrhœa, on April 4; on May 22 Mediterranean Fever was diagnosed. This was almost certainly contracted during his preceding seven weeks' sojourn in hospital. Only one case of fever occurred at Ricasoli (February 3) during the first four months of the year.

Case 17.—Private Knight, Hants Regiment, was admitted from Couvre Porte to Cottonera, No. 1 Ward, on March 28, with gonorrhœa. Mediterranean Fever was diagnosed on May 23, seven weeks afterwards. Infection was almost certainly contracted in hospital.

Case 18.—Private Wilkinson, Lancs. Fusiliers, was admitted from Zeitun Barracks to Cottonera, No. 1 Ward, with gonorrhoea on March 8: the first symptom of fever was felt about June 8, and the diagnosis made on June 13. Infection certainly contracted in hospital.

Case 19.—Private Shortland, Hants Regiment, was under treatment in Cottonera Hospital for gonorrhoea from April 26 to June 3. On June 20 he fell ill, and was admitted on 21st, suffering from fever, which was diagnosed on 29th. The shortest incubation period may probably be considered to be about 14 days; he might have contracted infection, therefore, in the three or four days immediately after his discharge from Cottonera on June 3; or while in hospital during the previous fortnight. Four cases were admitted from Verdala Barracks in June, one being from the same company as Shortland; there had been no admissions in May; during this month and the early part of June, when he presumably contracted infection, there were 20 or more cases of Mediterranean Fever in Cottonera Hospital, which was therefore a more likely source of infection than Verdala Barracks.

Case 20.—Gunner Taylor, 99th Company, R.G.A., was under observation for mental disease in Cottonera Hospital from June 2 to 20; he was readmitted with fever on June 29, and Mediterranean Fever was diagnosed July 7. It is more likely that infection was contracted between June 2 and 20, than between June 20 and 29; or before June 2.

During July and August no cases of Mediterranean Fever appear to have arisen among the patients at Cottonera, though four orderlies of the R.A.M.C. were attacked.

Case 21.—Private Smith, Royal West Kent Regiment, was in the hospital from June 27 to July 5, and from July 7 to August 5, suffering from wound of foot: during the latter period he was in No. 1 Ward. He was readmitted with gonorrhoea on August 11. He first felt ill with fever on September 8, and Mediterranean Fever was diagnosed on September 9. He might have contracted the infection during his brief residence in Floriana Old Barracks between August 5 and 11; or in hospital during the 24 days immediately preceding the onset of his illness, when he was in No. 1 Ward, in which were Mediterranean Fever patients. Floriana Old Barracks suffered from fever earlier in the year, but no case was admitted from them between July 24 and August 28.

Case 22.—Private Palmer, Essex Regiment, was transferred from Valletta to Citta Vecchia Sanatorium on December 19, 1904, suffering from hernia; on February 2, 1905, his "disease" was changed to Mediterranean Fever; the date of onset is not certain, but the probabilities are that infection was contracted in hospital, either at Citta Vecchia or Valletta.

Case 23.—Gunner Haynes was admitted to Forrest Hospital from Tigne on May 12, with enteritis, and was transferred to Citta Vecchia on August 16; on September 1 his "disease" was changed to Mediterranean Fever; the blood had reacted before leaving Forrest, and infection was, without doubt, contracted there.

Of the above 23 cases it may be affirmed that 16 almost certainly became infected in hospital; Cases 5, 6, 10, 14, 19, 20, and 21 are doubtful: but in my opinion the probabilities are much in favour of hospital infection in all the cases except No. 5 (Bush), 10 (Heaton), and 14 (Abbott), in which the uncertainties are considerable. Of the 11 Valletta cases, eight were venereal patients treated in 20 B Ward; Salmon and Wilding had been inmates of 20 A Ward; in the case of

Heaton, who had been under treatment for debility, the ward is uncertain. Of the 10 Cottonera cases, four venereals and one other (Smith) had been treated in No. 1 Ward, two (Abbott and Taylor) had been in observation wards, two (Collis and Haines) in the "fever wards," and one (Bishop) in No. 5 Ward. Therefore, 10 out of the 11 Valletta cases had been inmates of the same apartment (albeit a very large one) as was occupied by patients suffering from Mediterranean Fever: and 3 out of the 10 Cottonera cases had, in the same way, been treated in the wards along with the Mediterranean Fever patients.

Of the 19 cases amongst N.C.O.'s and men of the R.A.M.C. at Valletta Hospital, the following were brought into intimate association with the Mediterranean Fever patients, being employed in the fever wards, either as nursing, or as general duty, orderlies: Elsey, Brooks, Smith (14,901), Bowden, McGill, Smith (19,123), McConaghey, Whitmore, Aldous, Hardless, Playle: 11 in all. The following were not employed in these wards, and did not come into any continued or close association with the fever patients. Q.M.S. Dudman, Brown (clerk), Farr (cook), Sergeant Dewberry (laboratory), Corporal Hughes (day wardmaster, not in fever wards), Corporal Woods (pay office), Robinson (P.M.O.'s clerk), Q.M.S. Bridges: eight in all. Of these eight men it may be said that not only were they not brought into any special contact with fever patients, but that they had absolutely nothing to do with them, either directly, or indirectly (except the two quartermaster-sergeants). These two non-commissioned officers had certain duties in regard to the clothing and bedding of the patients that would constitute an indirect connexion. Sergeant Dewberry was specially employed as assistant in the laboratory of the Mediterranean Fever Commission; there can be little doubt that it was in this occupation that he contracted the disease.

All the corporals and privates of the R.A.M.C. sleep in the same barrack room, No. 31 (with an adjacent bunk); this is a large apartment, 96 feet long by 31 feet wide, the side annexe being $28 \times 17\frac{1}{2}$ feet. The room is well lighted, and airy in appearance, but on account of its extreme width and the absence of through cross ventilation (the annexe and two sergeants' rooms adjoining it on one side), it is difficult to secure a satisfactory change and renewal of the contained air. The height is 20 feet in the main room, and 15 feet in the annexe. The accommodation is authorised as for 54, giving an average cubic space of 1238 cubic feet per head; or, reckoning the height at 12 feet, of 770 cubic feet per head. The room has been full throughout the year, but not overcrowded in the hot weather, as many of the men sleep out on the roof, or on the verandah. The three men attached to R.A.M.C. for duty also slept here. Of these, two (Davis and Franklyn) were employed in the female hospital in general duty work. Cases of

Mediterranean Fever have been under treatment in this hospital throughout the year, but the orderlies were not brought into direct association with them in any way.

Of the seven cases occurring amongst R.A.M.C. at Cottonera Hospital, five were employed in the Mediterranean Fever wards; the other two, Rogers and Miller, were both employed in the hospital kitchen; part of their duties being to supervise the milking of the goats; they also took their turn of general night duty.

The two cases of R.A.M.C. at Citta Vecchia had both been employed in attendance on convalescent Mediterranean Fever patients, of whom there have been a large number at this hospital throughout the greater part of the year.

At Valletta Hospital four ladies of Queen Alexandra's Imperial Military Nursing Service have been employed in nursing duties. Of these, one has suffered from Mediterranean Fever during the past year. At Cottonera there is also a staff of four, and during 1904 and 1905 six nursing sisters have been attacked; four cases occurred in June and July, 1904, and two in January, 1905. At the Military Families Hospital, which is situated alongside of the military hospital, Valletta, the head nurse was placed on the sick list with Mediterranean Fever on June 20, 1905. All these ladies have been engaged in attendance on patients suffering from the disease, and have, therefore, been brought into intimate contact with them. At Valletta the sisters live in the hospital quadrangle. At Cottonera there are sisters' quarters in a detached house in the hospital grounds. It has been the practice until July, 1905, to milk the goats that provide milk for the patients on a plot of ground within a few yards of these quarters; and there is no doubt that this area was extensively fouled every day for a long period. No cases have occurred amongst the sisters at Cottonera during the summer, since the goats were removed from this spot.

§ 8.—*Women and Children.*

During the period under review there have been, as far as I have been able to ascertain, 38 cases of Mediterranean Fever amongst the families of the troops, 27 of which have been women, and 11 children. The exact number of the population from which these cases were derived is not yet available; but probably varied little from that of the preceding year, when there were 567 women and 928 children present in Malta (belonging to the garrison) on an average. The cases occurred all over the island, and there was no particular prevalence in any one group of quarters.

Allusion may be made in this place to the very remarkable prevalence of Mediterranean Fever in the New Misida Married Quarters in 1904. These quarters accommodate 44 families; A Block was completed and

taken into use in 1903, B Block in 1904. A study of the cases shows that there were 12 cases in 44 families, of which nine at least occurred in the 24 families occupying A Block. Twelve of these quarters are on the ground floor, and 12 on the upper floor; one of the cases occurred in No. 3 on the ground floor, and eight cases occurred in the 11 quarters on the upper floor. All the adjoining quarters were affected, from Nos. 14 to 19 at the north-east end of the block; Nos. 20 to 24 were unaffected, and in B Block to the south-west, there were two cases only amongst 20 families. It is uncertain whether Sergeant Biltcliffe lived in A or in B Block.

During the year 1904 there were, according to the Annual Sick Return, 109 cases of Mediterranean Fever amongst the women and children of the garrison. I have only been able to trace records of 67 of these, viz., 42 women and 25 children; the remainder were probably treated in quarters, and as to the diagnosis, I am unable to offer any opinion. Without attempting any statistical statement as to the prevalence in the various married quarters, it is certainly the case that nowhere else was there such an alarming incidence as in this particular block of buildings.

There are not many instances in which more than one member of the same family has been attacked. The following are all that I have been able to ascertain as occurring during 1904 and 1905. In the Misida quarters, Floriana, Mrs. Sanders was taken ill in July, 1904, and Colour-Sergeant Sanders was admitted on August 30; Sergeant Rogers was admitted September 21, and Mrs. Rogers on December 13; Mrs. Westbrook was placed on the sick list on October 2, 1904, and a child on January 23, 1905, the mother having a relapse a few weeks later.

At the Camerata married quarters (where about 90 families are in occupation) only one instance has occurred lately: Staff-Sergeant Lowe was taken ill in September, 1904 (presumably infected in hospital), and Mrs. Lowe in October. At St. Francis Ravelin Corporal Sullivan was admitted on July 13, and his child fell ill very shortly after. Two children in the family of Sergeant Hammett were attacked in 1904, one in June, the other in October. At Valletta Hospital, the wife of Quartermaster-Sergeant Bridges was admitted on May 27, 1905, the child on July 28, and Sergeant Bridges himself on August 11. Quartermaster-Sergeant Dudman was admitted on January 8th, 1905, and Mrs. Dudman on July 17. Conductor Fasson and Mrs. Fasson, living at Sliema, were placed on sick list on April 23 and May 9 respectively. The wife of Captain Challoner, living at Sliema, was first taken ill in November, 1903; the illness continued until May, 1904; on July 1 her son sickened, and a fortnight later her daughter. The wife of Major Preston, also living at Sliema, was attacked in the middle of January, 1905; her sister, and an English maid, were

both taken ill about four weeks later; Gunner Hardy, a soldier servant living in the house, was admitted to hospital on April 18.

In some of these cases some common condition was most probably the cause of the attack in both man and wife, or parent and child; but where an interval of several weeks elapses between the attacks, the likelihood of direct infection must be borne in mind. But as pointed out by Dr. Johnstone in his Report of last year (p. 38), if direct infection were always an important factor in the spread, it would be expected that a large proportion of multiple attacks in families would occur, and this has not been the case.

SECTION III.

From the foregoing account of the mode of prevalence, or behaviour, of the Mediterranean Fever epidemic during the first nine months of 1905, it may now be possible to gather some outstanding facts that will help either (1) to indicate the mode, or modes, of spread of the infection; or, if this is not evident, or probable, then (2) the conditions that assist in the spread of the disease may be ascertained, or shown to be probable.

§ 1.

It has been shown (1) that Mediterranean Fever has appeared in all the barracks in the islands, in which are quartered any considerable body of men (say one hundred or more).

(2) Although the disease has been universal throughout the garrison, the barracks have been affected very unevenly, Lower St. Elmo having had an attack ratio of 137 and Ricasoli of only 23 per 1000. The highest incidence has occurred in Valletta Hospital, 143 per 1000; Lower St. Elmo, 137 per 1000; Cottonera Hospital, 116 per 1000. These three places have suffered far more than any others, Upper St. Elmo having only had 66 admissions per 1000, and all the other barracks being less affected (mostly between 40 and 60), until at the bottom of the list come Verdala (27), Ricasoli (23), and Ghain Tuffieha Camp (8 per 1000).

(3) All the different bodies of troops have suffered, except such small parties as the Military Foot Police (numbering 16), and the Mounted Infantry Staff (numbering 11); and one battalion of over 500 strength, but which left the island in March (K.O. Yorkshire Light Infantry).

(4) The variation in prevalence has been equally well marked in the case of different bodies of men, as in the case of different barracks. The Royal Army Medical Corps suffered to the extent of 193 per 1000; the Hampshire Regiment had only 26 admissions per 1000. After the R.A.M.C. the Essex Regiment suffered most, 88 per 1000; four other infantry battalions and the Royal Artillery had between 43 and 54,

while the Royal Engineers had only 33, and the Hampshire Regiment only 26 per 1000.

(5) Although the total number of admissions for Mediterranean Fever increased in March, and again very markedly in May, remaining with little variation at a high level throughout the rest of the summer, this was not the case uniformly throughout the island; the maximum prevalence differing in different barracks; *e.g.*, in Lower St. Elmo it was in June, at St. George's in August, and at Cottonera in July.

(6) On examining more closely into the prevalence of the disease in different regiments it is found that there is a considerable unevenness of incidence on different groups, *i.e.*, companies, occupying the same barracks, and living under apparently almost identical conditions. For instance, in the Essex Regiment, G and H Companies had each 19 cases; of these 38 cases, 21 occurred between May 8 and July 8; alongside of them, and living under the same conditions in every way, were D and F Companies; no case at all occurred in D, and only one in F Company, during this period. The difference (or, a difference) between the two bodies was, that G and H had been living in Lower St. Elmo all the year, while D and F had been at Gozo until May 8. Again, from the four barrack rooms in the middle storey, Nos. 9 to 12, there were admitted 16 cases up to the departure of the regiment on July 8; from rooms Nos. 9 to 12 on the upper storey, accommodating the same number of men (about 90), there were admitted in the same period only six cases; in the former case the rooms were occupied throughout the whole time by H Company; in the latter the rooms were occupied by E Company up to May 6, who then went to Gozo, their place being taken by F Company, from Gozo. Sixty-four cases have occurred in the four companies that have been all the time at Lower St. Elmo and Imtarfa, 17 cases in the other four companies that have been part of the time at Gozo, as well as at St. Elmo and Imtarfa. The great prevalence in the Essex Regiment appears to be connected with residence in Lower St. Elmo barracks, and especially with certain rooms in those barracks.

(7) The Lancashire Fusiliers took the place of the Essex in Lower St. Elmo on July 11; after this date seven cases occurred in C Company, which occupied the rooms vacated by G Company of the Essex, the company that had suffered severely in the earlier part of the year. In H Company of the Lancashires, seven cases occurred after arrival at St. Elmo; they occupied the same rooms as F Company of the Essex, a company which had arrived from Gozo in May, and had suffered little; but shortly after their arrival at Imtarfa they had three cases (presumably contracted while living in these rooms at St. Elmo); local infection seems not unlikely. The other companies of the Lancashires suffered little.

(8) The Dublin Fusiliers have occupied St. George's Barracks since

March, 1904 ; all the companies are living under precisely similar conditions ; out of 41 admissions for Mediterranean Fever during the nine months, A Company has had 13 and H Company has had only one : these two companies live in the same barrack block, use the same cookhouse, latrine, urinal, and ablution rooms ; they are of the same strength ; yet one has had 13 cases, the other only one.

(9) The eight different companies of the Royal Artillery have suffered very unevenly ; No. 65 Company has had 83 per 1000 admissions, No. 63 company only 18·5 per 1000. The two companies stationed at Upper St. Elmo have had many more cases in proportion than the three companies at Ricasoli ; Upper St. Elmo has had a greater incidence than any other barrack except the adjoining Lower St. Elmo ; Ricasoli has had the lowest incidence of any barrack.

(10) The Royal West Kent Regiment, occupying Floriana barracks, have not suffered severely ; they furnish another example of an uneven, and limited, prevalence ; of 35 total admissions the origin of one is uncertain ; as to the remaining 34, the *Old Barracks* (accommodating 360) contributed 23, the remaining 11 coming from the *New Barracks*, Ravelin, etc. (accommodating 563).

(11) The ratio of incidence on Hospital populations, reckoning patients and attendants together, is high, 104·7 per 1000, but not so high as among the troops at Lower St. Elmo (137 per 1000) ; at Valletta Hospital, however, it is 143 per 1000, Cottonera showing 116 per 1000, and the smaller hospitals much less. The attendants, taking all the hospitals together, suffer much more (193·5 per 1000) than the patients (60·5 per 1000) ; but the incidence upon the patients is not fairly comparable, as they are a very fluctuating population. Taking the two large hospitals, the incidence upon the orderlies of the R.A.M.C. is 257 per 1000 at Valletta, and 156 per 1000 at Cottonera, both figures being considerably higher than in any other body of troops.

(12) There has been no great prevalence of Mediterranean Fever amongst the married families during the period under consideration, though cases have occurred everywhere throughout the married quarters. In comparatively few instances have two or more members of a family been attacked under circumstances indicating direct infection from one to the other. There has been no recurrence of the remarkable outbreak of 1904 in one particular set of newly-built married quarters (Misida Bastion), which seemed to point so strongly to some strictly localised condition.

§ 2.

We may now consider what information can be obtained from the foregoing account of Mediterranean Fever prevalence in 1905, as to its probable mode of spread. Infected water, infected food, infected air,

are the three most obvious possible channels of conveyance ; these may be first dealt with.

i. *Infected Water*.—The water supply of Malta has been sufficiently described in Dr. Johnstone's Report. So far as concerns the military population, there are no barracks in which there is not an ample supply of drinking water of good quality (known as No. 1 Water), in every case laid on direct from the main, and therefore free from any danger of local contamination. The same water is supplied to all the barracks. It is inconceivable that with such a supply, and such a method of distribution, there should be a prevalence characterised by the special features that have been above noted, if the drinking water were the channel of conveyance. Although it is the case that another quality of water is also supplied for ablution purposes, which is not so pure, and which therefore might be thought to be a carrier of infection, this is in the highest degree improbable. Everywhere the taps supplying this water are marked, "Not for drinking," and everywhere the pure supply is quite as readily obtainable as this ablution water. Careless as the soldier may be about his health, he is not such a fool as to drink water marked "unfit," when there is a tap of good water alongside ; such a case might occur very exceptionally, but not as a common practice. Moreover, in barracks that are supplied *only* with No. 1 water, such as St. Andrew's and Tigne, and in married quarters, such as the new Misda blocks at Floriana, Mediterranean Fever has prevailed more extensively than in some other places, such as Verdala and Imtarfa, which have a double supply. As was pointed out by Hughes, if the inferior water supply were the channel of conveyance, "we should expect the inhabitants of private houses in the same area supplied only with the good water to be immune from [Mediterranean] fever ; but this is very far from being the case."

The use of *ice*, and of *aërated waters*, is really to be considered along with the question of water supply. During the summer months the use of ice is universal amongst those that can afford it ; few people in comparison drink plain water ; nearly everyone drinks aërated water and ice ; if any particular parcel of ice were infected there could hardly fail to be an explosive outburst of the fever, analogous to a water epidemic. There is no evidence to this effect, and no suspicion seems ever to have been excited that such was the cause. So with lemonade, soda water, and other aërated drinks that are consumed by everyone in the hot weather, there appears to be no evidence whatever incriminating these articles.

§ 3.

ii. *Infected Food*.—The two articles of food that appear to be the most likely channels are *milk* and *uncooked vegetables*. With regard to the latter, I regret that I have no evidence whatever : the methods of

cultivation in vogue in Malta would lead one to look upon all uncooked vegetables as dangerous articles of food. Human excrement is largely used as manure, and one would regard lettuces, tomatoes, radishes, and all vegetables eaten in the way of salad—*i.e.*, uncooked, also certain fruits, such as strawberries, with great suspicion. There is a fairly general consumption of such articles by the people who can afford to buy them; but by the troops they are hardly eaten at all. It is a failing of the British soldier that has been frequently commented on, that he does not take advantage of the vegetable food that is available, wholesome, nourishing, and cheap, in the different parts of the world in which he serves. He has no culinary instinct, he cannot dress vegetables, and he cannot make a salad. Such things do not form part of the men's ordinary food, nor, as far as I have been able to ascertain, are they an article of consumption in the regimental coffee shops and supper bars, or in the eating-houses frequented outside barracks.

§ 4.

The question of the conveyance of *milk* is one of great importance, on account of the discoveries recently made as to the existence of Mediterranean Fever in goats, and the presence of *Micrococcus melitensis* in milk of apparently quite good quality, and yielded by goats in apparently perfect health.

The facts bearing upon this question now to be related fall under three heads: (1) The conditions as to milk supply of the different bodies of troops in Malta: (2) the conditions as to milk supply of the married families, and the prevalence of Malta Fever amongst them; (3) evidence as to milk consumption by Malta Fever patients.

(1) *Conditions of Milk Supply amongst the Troops.*

The procedure adopted in the different regiments has been as follows:—

Royal Garrison Artillery—Upper St. Elmo: 65th and 96th Companies.—Only condensed milk is used; no goats at all come into the fort, except one or two for the married people occupying the two married quarters.

Tigne: 1st, 99th, and 102nd Companies.—All milk used by the troops is tinned milk, except at the sergeants' mess, where it seems that a small quantity of goats' milk has been used.

Ricasoli: 5th, 63rd, and 100th Companies.—All milk used by the troops is condensed milk; one or two goats come into the fort for the married families.

Oullying Forts.—Only condensed milk is used.

This custom of using condensed milk is, in Malta, almost peculiar to the Garrison Artillery; it is probably due to the fact that a great

number of the men have had considerable service in India, where the milk is, speaking generally, of obviously poor quality from a house-keeper's point of view, and lies under a very widely-held suspicion of impurity from the medical standpoint, chiefly in regard to enteric fever. This is now a matter of general knowledge amongst the troops in India. Condensed milk is now of such good quality, and so cheap, that the Artillery and their families use it, almost without exception, and are well satisfied with it. In the outlying forts, where the arrangements and supervision are less complete, goats' milk may be occasionally used; but I think it must be of rare occurrence, because amongst the Artillery the feeling is, and for a long time has been, decidedly in favour of condensed milk; the kinds used are Milkmaid brand and Nestlé's.

The *Lancashire Fusiliers* have used goats' milk. Since arrival at Lower St. Elmo on July 11 no goats have been allowed inside the fort, except one or two for married families. The goats have been milked outside, morning and afternoon, the milk brought to the regimental cookhouse and immediately boiled, under the supervision of the master cook.

The *Royal Sussex Regiment* at Imtarfa use only condensed milk.

The *Hampshire Regiment* (at Verdala) use goats' milk; the goats are milked outside the barracks; the milk is brought to the cookhouse and boiled.

The *Essex Regiment* use goats' milk. When at Lower St. Elmo no goats were allowed inside the fort, except one or two for the married families. The goats were milked outside, the milk brought into the regimental cookhouse, and boiled under the supervision of the master cook. The same arrangement is carried out at Imtarfa.

The *West Kent Regiment* at Floriana use goats' milk. The goats are milked outside the barrack gate. The milk is then taken to the various cookhouses (Old Barracks, Notre Dame, New Barracks), but it has not been boiled during the greater part of the summer.

The *Dublin Fusiliers* at St. George's use goats' milk. The goats are brought to the skittle-alley in the lines, and milked under the supervision of the master cook, who is then responsible that the milk is boiled in one of the company cookhouses. It is all boiled together. The skittle-alley is cleaned out daily.

The *Rifle Brigade* at St. Andrew's use goats' milk. The goats all come to one cookhouse, and are milked under the eye of the master cook, who then sees that the milk is boiled. One or two goats are sent up to the married quarters.

At *Valletta Hospital* the goats are milked under supervision in the paved back entrance to the lower square. The milk is "Pasteurised" in an Aymard steriliser.

At *Cottonera Hospital* the goats are milked under supervision in a

specially selected place. The milk is "Pasteurised" in an Aymard steriliser.

At Forrest, Imtarfa, Citta Vecchia, and Gozo Hospitals the milk is boiled.

The rule has been to boil, or Pasteurise, all milk throughout the garrison, with the exception of the West Kent Regiment and the Royal Artillery; the last named have used only condensed milk. As regards hospitals, this rule has been in operation for the whole of 1905 and most part of 1904. As regards troops, it is difficult to state exactly when the boiling commenced. Until recently, it was looked on as an advisable proceeding, but perhaps hardly worth the trouble, and was probably carried out somewhat perfunctorily. But, from the beginning of July, 1905, there can be very little doubt that, with the exceptions mentioned, all milk consumed by the troops in barracks has been definitely boiled, that is, "brought to the boil." Horrocks has shown that an exposure for 10 minutes to 68° C. (154° F.) is sufficient to destroy *Micrococcus melitensis*, naturally present in goat's milk (i.e., the milk of a goat that is suffering from the fever and excreting the organism in its milk). Therefore, even supposing some laxity in the carrying out of the boiling regulations, the milk supply of the troops (with the exceptions noted) must be regarded as having been rendered harmless. So much attention was drawn to the question of the milk supply (consequent on the discovery of the presence in the milk of *Micrococcus melitensis*) during June, 1905, and the early part of July and the admissions for fever were so numerous throughout the garrison, that I feel no doubt that the boiling was carried out effectively, and not perfunctorily, from this time onward.

No diminution in the number of admissions occurred in August or September, when the full effect of this precaution would have become evident; on the contrary, the admissions increased from 67 in July to 86 in August, and in September numbered 77. The regiment that did not boil its milk (the Royal West Kent at Floriana) had an admission rate for the nine months of 43.53 per 1000, being the lowest but three of any corps in the island.

It may have been that men were infected by milk consumed outside barracks. This cannot be denied, but it is highly improbable. It is very rarely that the British soldier drinks milk at any time, and the refreshment of which he partakes during his hours of relaxation outside barracks is almost invariably of an entirely different description.

(2) Conditions of Milk Supply among Married Families.

A house-to-house visitation was made throughout the various married quarters, and particulars were obtained of the people's habits in this matter; the results of which are summarised in the following

paragraph. The figures refer to 1904 and 1905. I am aware that statements made in answer to questions of this kind have to be taken *cum grano*, but I feel confident that the actual state of things was ascertained in the great majority of cases, practically in nearly every case. Moreover, no instance has been put down as positive, *i.e.*, no use of condensed milk only, or of condensed and boiled milk only, has been returned as such, unless there was good reason to believe that this was really the case: any case of the least doubt has been returned under the heading of "unboiled, more or less."

Out of the 322 families thus inquired into, embracing a total population of 1213, it is seen that 441 persons consumed only condensed milk, and among these 14 cases of Mediterranean Fever occurred, giving a ratio of 31·74 per 1000. Amongst 398 persons, who drank either condensed or boiled milk, but never unboiled milk, 13 cases occurred, giving a ratio of 32·66 per 1000, which is practically identical with the first-mentioned. Taking these two categories together, we have a population of 839, with 27 cases, *i.e.*, a ratio of 32·18 per 1000. The remainder, 374 persons, who drank unboiled milk either habitually or occasionally, furnished 24 cases, *i.e.*, in an attack ratio of 64·17 per 1000, or exactly twice the incidence of the protected population. Taking all the men together, 10 cases occurred among 322, or 31·05 per 1000. All the women, 322, had 21 cases; all the children, 569, had 20 cases. The ratio for the whole population (51 cases among 1213 persons) is 42·04 per 1000. The women suffered the most, 65·21 per 1000; the men the least, 31·05 per 1000; the children very slightly more than the men, 35·14 per 1000.

If, for the sake of argument, we leave the men out of the question (for the importance of milk as a factor in causation is likely to be much less in their case than in the case of women or children), and deal only with the remaining population of 891, we find that 277 persons drinking unboiled milk furnished 21 cases (75·81 per 1000), while 614 persons drinking only boiled or condensed milk furnished 20 cases (32·57 per 1000).

If we consider the children only, as being those most likely to be affected, we find that 180 drinking unboiled milk furnished 13 cases (72·23 per 1000), while 389 drinking boiled or condensed milk furnished seven cases (17·99 per 1000), that is, the former suffered just four times as much as the latter.

The numbers are too small to prove anything, but there is, in my opinion, a considerable presumption that, in the cases occurring amongst women and children, the disease was introduced by infective goats' milk. It is to be noted that a disproportionate number of cases occurred in the Floriana married quarters (17 cases in 63 families, with a population of 230; three other women and one other man were attacked, but their milk supply is not known). As stated elsewhere

(Section II, § 8) there is reason to believe that some special cause was in operation in these quarters in 1904. Looking at the prevalence of the disease amongst the families as a whole, in spite of all the variations in the surroundings of the quarters, in their structure and sanitary fittings, the character of the milk supply appears to have an important, and in the case of children, a dominant influence.

(3) Particulars have been obtained in regard to 155 cases of Mediterranean Fever that have occurred amongst the troops in 1905, as to their consumption of milk before being taken ill. What the men usually say is, that they "drink no milk at all"; on further questioning this resolves itself into "no milk except in tea."

In 13 cases it was definitely acknowledged that unboiled milk had been drunk, in greater or less quantity, as a beverage; in nine cases that it had been taken in tea only. In these 22 cases infection by milk is a quite possible explanation of the causation of the disease.

In 26 cases it was definitely stated that no milk *at all* had been drunk, not even in tea; or if any had been taken in tea, that it was condensed milk; and in 17 cases it was stated with equal definiteness that, although milk had been taken in tea, it was known to be boiled. In these 43 cases infection by milk must be regarded as in the highest degree improbable.

Three men stated that they had consumed a considerable quantity of milk, but that it had always been boiled, or "sterilised." These three were cases that had arisen in Valletta Hospital, where an Aymard's steriliser has been in use for two years, and the statements may be taken as correct.

The remaining 87 cases stated that they drank no milk at all except in tea; whether or no it had been boiled they were not aware. It is the universal custom in barracks to add milk to the tea in the cook-house, before distribution; individuals, therefore, would not know whether it had been boiled or not. The practice of boiling the milk became general at the beginning of July, 1905:* any men admitted after the end of this month, if their milk consumption was confined to the regulation tea, are not likely to have drunk any milk other than what had been boiled. This applies to 34 cases. To these must be added seven cases in the Artillery (who use no goats' milk at all). Deducting ($34 + 7 =$) 41 from the 87 cases, there are left 46 cases, as to whom it may be said that infection by milk cannot be excluded.

It is seen that, out of the 155 cases, milk infection is quite possible in 22, and is not unlikely, or at any rate not to be excluded, in

* The West Kent Regiment did not boil their milk until later; only three of these cases belong to this regiment, and they were admitted before June; the omission of the precaution did not apparently bring about any cases in this regiment during July and later, any more than its adoption prevented their occurrence in the other regiments.

46: while it is unlikely in 41, and in the highest degree improbable in $(43 + 3 =) 46$. I regret that I have been unable to interview every one of the 487 cases that have occurred during the period under review. All the cases that I was able to get access to I did examine, with the result just stated. I have no reason to doubt that they present a fair sample of the whole; but the account is, of course, not a complete one, referring only to about one-third of the cases that occurred. If trustworthy information could be obtained as to 500 cases, the question might be settled. As it is, I consider that while the evidence as regards married families, and especially children, is fairly strong in favour of the transmission by milk, as regards the troops it is negative. It would not be justifiable to affirm that the circumstances of the milk supply of the troops, considered in relation to the fever prevalence, in any way invalidate the theory of milk-transmission; but I do not find anything in these circumstances, as they existed during 1905, to lead one to suppose that milk can have had any important part, or indeed any part at all, in disseminating the specific poison during this epidemic *amongst the troops*. The experimental evidence obtained by the laboratory investigations of the Commission during the past year have been so conclusive as to the infectivity of goats' milk in Malta, that no want of proof from the epidemiological side can weaken its force; all that can be said is that milk does not explain the incidence of the disease upon the troops during this particular period. That a body of men such as the Royal Artillery, numbering about 2000, should have had 88 cases (45 per 1000), although practically they drink no goats' milk at all; that of these men some companies (such as those at Upper St. Elmo) should have had an attack rate as high as 66 per 1000; while the general attack rate has been 53, in one regiment only 27, and in a regiment that habitually partook of unboiled milk not more than 45 per 1000 (exactly the same as in the Artillery who drank none at all)—this indicates that milk infection has not been an important mode of propagation among the troops.

A rational explanation of this lies in the fact that, as already mentioned, milk enters but very slightly indeed into the dietary of the British soldier. Occasionally and exceptionally the reverse is the case; and it is not at all unlikely that the men who have been in the habit of drinking milk (as a beverage or food, not merely in tea, etc.), have suffered largely. There are, however, no statistical data in existence as to the frequency or rarity of milk-drinking amongst soldiers. It is impossible, therefore, to say whether or no these milk-drinkers have suffered disproportionately in Malta.

§ 5.

iii. *Infected Air*.—Recent researches have shown (1) that *Micrococcus melitensis* is discharged in the urine of Mediterranean Fever patients, being frequently present in enormous numbers; (2) that it is able to survive in a dry state for long periods when not exposed to the direct rays of the sun; (3) though it has not been demonstrated in the fæces of patients, Eyre has found it in the fæces of artificially infected guinea-pigs. There is, therefore, good ground for supposing that air containing excretally contaminated dust may bring about transmission of the disease. Such air may be “sewer air,” or “latrine air,” or “urinal air,” or (in Malta) the air of houses, streets, roads, and fields throughout the islands.

(a) The well known observations of Carnelley and Haldane, Parry Laws and Andrewes, Petri, and others, have shown that the air of sewers, which are regularly and properly flushed and ventilated, is remarkably free from micro-organisms of any kind; moreover, those that are present are derived from the external air rather than from the contents of the sewer. When fermentative or putrefactive processes occur, however, with formation of gas bubbles, there is a likelihood, as Frankland has pointed out, that sewage microbes may be disseminated in the air. Tichborne considered that they might be carried about, as on a raft, by condensed vapour formed during the cold hours of the night, and dissipated when the air becomes warmed, leaving the imponderable microbe floating in the air. There is such a large body of evidence connecting outbreaks of infectious disease with the breathing of air contaminated with sewer emanations that some such explanation is required; notwithstanding the observed paucity of micro-organisms (and *a fortiori* of pathogenic organisms) in the extensive experiments that have been carried out, the connexion between disease outbreaks and the breathing of sewer air, or excretally contaminated air, has also been a matter of such frequent observation that the possibility of transmission of disease in this way cannot be disregarded. It is known that bacteria cannot be given off from a surface that is kept constantly moist; from a surface that is alternately moist and dry, however, they would be likely to be dislodged by various causes, such as concussion, strong currents of air, or even in the course of drying. The sewerage system of Malta has up to the present suffered from a very insufficient supply of flushing water; and there is no doubt that, to a very great extent, the sewer walls have been alternately wetted and dried, and therefore in a condition to render the disengagement of sewage organisms, including the various pathogenic bacteria present in the excreta of infected persons, not only possible but likely. Amongst these pathogenic bacteria *Micrococcus melitensis* must be considered to be potentially present for a great part of the year.

In regard to barracks this inadequacy of drain flushing has also existed to a greater or less extent. At Upper and Lower St. Elmo, Floriana, St. Francis, Manoel, Tigne, and Ricasoli the amount of flushing water has been on the whole sufficient, and the condition of the drains satisfactory; that is to say, they have been self-cleansing, and their walls have been free from deposit. At St. James' Cavalier, Verdala, throughout the Cottonera Lines, and at St. George's, Pembroke, the flushing water has been, as a general rule, throughout the first nine months of 1905, scanty; it is probably correct to say that it has been insufficient for the proper cleansing of the drains. At Imtarfa it has been somewhat scanty, and at Gozo. St. Andrew's has only been taken into occupation during the summer of 1905; so far there has been a sufficient supply of flushing water. There has been no scarcity of flushing water for the drains at any of the hospitals. Throughout all these barracks the drainage systems are, on the whole, of modern type, well laid, well ventilated, and well trapped; only once did I find any serious obstruction. There are however numerous defects of detail in construction or maintenance which require attention (as specified in a separate report), and which, unless attended to, will in course of time lead to dangerous conditions in the barrack drainage systems. As especially bearing on the point now under consideration—escape of sewer air—may be mentioned the following:—(1) The unsealing of w.c. traps in several married quarters in Strada Magazzini, Floriana (owing to the quarters being vacant and the closets disused). (2) The unsealing of gully traps outside Married Quarters at Imtarfa, owing to no water being used for washing the verandahs (to receive which these traps were provided), and to long-continued dry weather. (3) Direct communication with a drain at the side of the road within a few yards of the back gateway of Verdala Barracks, an inlet acting as an outlet, and the drain being imperfectly ventilated, so that a bad drain smell is perceptible on a much-frequented roadway. (4) There has been persistent complaint on the part of the occupants of the Old Block of Married Quarters in St. Francis Ravelin, as to bad smells coming from the ventilating shafts of the Civil Government sewer, the nearest of which shafts is some 300 or 400 yards distant; alterations have been made from time to time, some shafts have been closed, and one has been carried to a greater height; the nuisance, however, still continues. (5) At Couvre Porte there is a ventilating shaft for the Civil Government sewer, opening over the roof of the barrack, and 42 feet above it; bad smells are complained of, especially at night.

The above five instances of escape of sewer air into, or in the neighbourhood of, barracks and quarters are the only definite cases that came under my notice in making my enquiries as to the sanitary condition of barracks in Malta. In the first instance, at the Strada Magazzini Married Quarters, Floriana, at the time of my visit a stoppage had

occurred in the main drain of the block of quarters ; I was informed that this was not an uncommon occurrence : as two branch drains at least enter the main drain at right angles, instead of in the direction of the flow, a stoppage is not unlikely to occur from time to time ; but I understand that on this occasion the actual obstruction was lower down, near to, or at, the junction with the street sewer. Such inspection pits as are provided are cemented down, so that the condition of the drain cannot be seen to and precautions taken to prevent a stoppage ; consequently it may exist for a day or two, or more, before being discovered ; meanwhile the foul air is laid on to the quarters, in which are situated the untrapped w.c.'s, and from them escapes into the small backyard, whence it gains access to the quarters above, whose windows open into this yard. Bad smells were particularly complained of by the occupants of No. 12 (Barrack Warden Budden) and No. 13 (Corporal Bellfield, Royal West Kent Regiment). Mr. Budden had an attack of Mediterranean Fever in June, 1905, his two children suffer from sore throat, not severely, but chronically. Corporal Bellfield's family have not suffered from any fever or throat affection. The wife of Corporal West (Royal West Kent Regiment), who occupied No. 6 quarter (in which is one of the faulty w.c.'s) the year before, was admitted for Mediterranean Fever in June, 1904. The occurrence of these two cases of the disease in connexion with the faulty sanitary condition is to be noted ; but no other cases have occurred in these quarters, nor have I been able to trace any connexion between the occurrence of Mediterranean Fever and the other instances of sewer air nuisance just mentioned. It is true that six cases have occurred during 1903-5 in the Old Block of Married Quarters, St. Francis Ravelin, which have been thought to be due to effluvia escaping from the civil sewer ventilator, some 300 or 400 yards distant. This, however, does not appear to be probable. Two cases were admitted from Couvre Porte in 1904, and one in 1905. This does not indicate any particular infective property in the emanations from the sewer ventilator above the roof of this barrack, undesirable as they undoubtedly are. Several cases of illness—often fatal—occurred during the summer amongst the children occupying the Married Quarters at Imtarfa, where drain air escaped through unsealed traps on to the verandah ; but none of these were Mediterranean Fever.

The theory of infection by sewer air of course presupposes that there has been previous passage of the specific *contagium* into the sewer in question. There is little difficulty in believing that this is the case in regard to the public sewers of Valletta, etc. ; but in the specific instances mentioned of drain emanations in barracks the same cannot be said ; there were but few cases of the disease at Verdala and Imtarfa, therefore little active contagion passing into the drains ; and it would be making too large an assumption altogether to put down these cases to

drain infection. In fact, the barracks where the flushing of the drains was most inadequate, such as Verdala, Cottonera Lines, and St. George's (in which therefore the drain air would be most dangerous) have not suffered the most severely; while the barracks that have suffered most (Upper and Lower St. Elmo, Tigne, and the two large hospitals) are those which, whatever their other sanitary shortcomings, have at any rate had no lack of water for flushing, no troubles with regard to their drainage arrangements, and certainly no defects leading to sewer emanations in the barracks.

The effect of breathing emanations from the public sewers in Valletta, etc., would be more likely to be evident in those who live in the crowded parts of this city, or of the other thickly populated places across the Grand Harbour. A plausible explanation would be thereby afforded of the high incidence on Upper and Lower St. Elmo, neither of which barracks can be approached without passing along crowded streets, whose inhabitants (mostly, though not all, of the poorer class) have little regard for any kind of sanitation, and are content that their closets and drains should be habitually foul and pestiferous. Verdala is however in almost equally bad case in this respect, yet it has suffered very slightly.

(b) *Latrine Air*.—The condition of the latrines in the different barracks in Malta is, in many cases, extremely unsatisfactory, the cause generally being an inadequate water supply. The type of latrine in general use is the "Jennings' continuous pipe latrine," which is a good pattern and, when properly used, quite free from offence. The dry earth system is still in use in some barracks. At Lower St. Elmo new water-closets were fitted up in the early part of 1905, with improved siphonic flushing arrangements; although these arrangements are susceptible of further improvement the latrines, both upstairs and downstairs, have been kept in a sanitary condition without difficulty, and the water supply has been ample throughout the year.

At St. Andrew's Barracks, only recently completed, the latrines are new, and of good pattern, and the water supply has been so far ample. At Fort Ricasoli, and at Valletta, Cottonera, Forrest, Imtarfa, Citta Vecchia and Gozo hospitals, the water supply has been sufficient, and the latrines kept in good order.

Throughout the rest of the barracks the latrines have not, speaking generally, been kept in a satisfactory condition. In the various barracks that make up the Cottonera Lines the supply of water for flushing has habitually been inadequate; indeed, it has often been altogether cut off, so that the latrine pans have been allowed to become partially dry, excreta remaining in the pans and fouling the sides, and in this way gradually drying up and becoming scattered about as dust; this occurred at Polverista, St. Paul's Bastion,

Vittoriosa, and elsewhere. At Upper St. Elmo, St. James' Cavalier, Floriana Old Barracks, and Verdala, the water supply has been scanty, and the flushing not done often enough; consequently, the latrines have habitually, or frequently, been over-full; in which case, when they are emptied, there is a likelihood of excreta remaining on the sides of the latrine to a greater or less extent, and eventually becoming dried and disseminated as dust. At Tigne a new latrine has quite recently been opened; at present it is in good order and quite clean. Tigne (until July, 1905), Manoel, Pembroke Fort, Imtarfa, Gozo, and all the outlying forts and encampments (except Fort Rinella and Camp Mellieha) have had the dry-earth system of removal; the removal is effected once only in the 24 hours, very early in the morning, about 3.0 or 4.0 A.M. As the greatest use of the latrines takes place between 8.0 and 10.0 A.M., it follows that the excreta are retained in the lines for from 16 to 18 hours every day instead of being removed at the earliest possible moment. If the application of the dry earth were immediate and thorough, this retention of foul matter might perhaps be harmless, or even inoffensive. As a matter of fact, it is very seldom the case that the system is properly carried out, and the net result is that dry-earth latrines are generally in a filthy and insanitary condition, for, at any rate, many hours of the day and night. All the latrines on this system in Malta (with very few exceptions) have been habitually in a foul state. Fort Rinella and Camp Mellieha have a water system of removal, with a good supply of water. Fort Ta Silch has had a dry-earth latrine for night use (which has also been habitually used during the day), and a temporary trench system just outside the fort; this arrangement has been very unsatisfactory.

The only barracks that can be considered to have been free from the effluvia of faecal matter are those first mentioned, viz., Lower St. Elmo, Ricasoli, and St. Andrew's; also Fort Rinella and Camp Mellieha; in all the others persons using the latrines have, during a great part of the day, been subject to whatever risks may be considered to arise from breathing air contaminated with faecal emanations, that is, effluvia from excreta in a more or less fresh condition. All the hospitals have been free from this risk. The case, as regards Mediterranean Fever in these barracks, is that the occupants of Lower St. Elmo have suffered more than those of any other barrack, and the occupants of St. Andrew's to about an equal extent with those of most of the barracks (see Table I); Ricasoli has suffered but slightly. The hospital population has suffered considerably, but this can hardly have been on account of the state of the latrines, which have been maintained in a satisfactory condition throughout the island.

In the two barracks that have suffered least, although in Ricasoli the latrine air has been free from faecal emanations, in Verdala the reverse has been the case: while of two barracks, lying alongside of

each other, and similar in situation and general construction, St. Andrew's, with quite new latrines well flushed, has suffered more than St. George's, with scantily-flushed latrines, many of which have been in existence for a long time, and have therefore become proportionately foul.

There does not appear, therefore, to be any definite evidence connecting the incidence of Mediterranean Fever with the presence of faecal emanations, as far as concerns the troops, during the period under review.

As regards married families, the principal Married Quarters—Camerata, Tigne, Old and New Floriana, New Verdala, St. Nicholas, Ricasoli, and St. Andrew's—are provided with water-closets of excellent pattern, and are quite free from any kind of drain or latrine emanations. This cannot be said of the older quarters, such as St. Nicholas Back, and the old St. George's blocks, where latrines of old pattern are still in use; nor of the hired quarters in Strada Magazzini, Floriana, which have been already alluded to. Although a few cases have occurred in the latter quarters, most of the women and children attacked have lived in the newer quarters (such as New Floriana), which are provided with water-closet chambers and fittings of the best and most modern kind. Such cases cannot be regarded as due to infection through "faecal emanations."

(c) *Urinal Air*.—The recent researches as to the viability of *Micrococcus melitensis* in dust, and the demonstration that Mediterranean Fever can be communicated to goats (though not, so far, to monkeys) by feeding them on dust infected with the urine of Mediterranean Fever patients, make it necessary to examine into the condition of barracks in regard to the presence or absence of urinary contamination of the air. Throughout the island the barrack urinals are constructed on the same general plan, viz., ranges of partitioned stalls made of slate, flushed with water from a sparge pipe, the flow being carried direct into the nearest drain. It has been for some years the custom to cover the slate surface with tar from time to time, a result of which has been that the surfaces have generally become rough and uneven, leading to collections of urinary sediment all over the lower part. The water flushing has also been quite inadequate to keep the stalls clean; this has been partly due to an insufficient quantity of water being used, and partly to its being inefficiently applied, the holes of the sparge pipes being very generally blocked up, or the pipes themselves being fixed in a wrong position. The consequence has been that barrack urinals have generally been dirty and ill-smelling. During the past year a new arrangement has been brought into use, according to which the water flushing is omitted, and in its place the urinal surface is coated over with a mixture of kerosine oil and lamp-black or tar. The best application for the purpose is a substance

called "heavy oil," but, as this has apparently not been procurable in Malta, various substitutes have been used in its stead: a mixture of colza oil and tar in equal parts, as used at Imtarfa, appeared to me to be the most effectual, and needed only to be applied once a week. No deposit takes place on the back of the stall, and there is not the least offensive smell. Elsewhere, results have not been so satisfactory. But when this, or some similar application, is used effectively, the urinals are undoubtedly cleaner than under the old arrangements. Although water must not be distributed over the surfaces to which the oil or tar has been applied, it is necessary to flush the *drain* with water, and to wash down the floor of the urinal frequently, otherwise the floor and the drain will become foul. This is what has occurred in practically every urinal in every barrack throughout the island; urine has been allowed to dry on the floor, and so become converted into dust and pollute the air.

With the knowledge that we now have that Mediterranean Fever often occurs in an ambulant and unrecognised form, there is little doubt that infective urine has been widely distributed throughout barracks in this way. On the other hand, it has been shown that exposure to direct sunlight destroys the specific organism in a few hours (Horrocks). In some barracks, as in Upper and Lower St. Elmo, the old barracks and bastions in Cottonera Lines, Verdala, St. Francis, Marsamuscetto, Old Laboratory, the old part of Floriana, and in the detached Forts, the urinals are under cover and shielded from the direct rays of the sun. This is also the case in all the hospitals. Under such circumstances *Micrococcus melitensis* might live for several days, as Horrocks has found that it will survive for 28 days in ordinary street dust, and in "building dust." In the newer barracks, such as Tigne, New Floriana, St. George's, St. Andrew's, and Imtarfa; also at Manoel, Ricasoli, Gozo, and, of course, in all the camps, the urinals are out in the open, without covering of any kind. The duration of infectivity of the dried urine would appear in these places to be very short indeed, almost negligible. It is certainly the case that the places which have suffered most, viz., Valletta and Cottonera Hospitals, Lower and Upper St. Elmo Barracks, have urinals that would retain infectivity longer than such barracks as Ricasoli, St. George's, and Imtarfa, that have suffered comparatively slightly. Also, Floriana New Barracks have had fewer cases proportionately than the *Old* buildings. But in the case of the two hospitals named much more care has been taken than in barracks generally to keep the floors of urinals clean, and in these places I do not think any appreciable risk of infection can have been incurred in this way. St. George's and St. Andrew's are identical in this particular respect, but have suffered differently; and Verdala, which has fared better than any other barrack but Ricasoli, has urinals as dark, and almost as confined,

as in any barrack. While there can be no doubt that every care ought to be taken to prevent fouling of the ground with urine, and to cleanse it frequently, when fouled, it can hardly be said that the fouling which has occurred affords any satisfactory explanation of the distribution of the disease during the recent epidemic.

There is another way, however, in which urinary infection may have been spread. None of the barracks in Malta are provided with night urinals. In every case the urine tub is still in use. In the older barracks it is placed on the floor outside the barrack-room door in the verandah or passage. In the newer barracks a special stand is provided. At Tigne New Barracks, St. Andrew's, and the new blocks at St. George's, the tubs are placed in an alcove behind the barrack-room, and, on the upper floor, have to be brought *through* the room, in order to be taken downstairs to be emptied.* It must often happen that urine gets splashed about on the floors. It is conceivable that infection might be spread in this way, and that the admission rate from these new, and in most respects sanitary, barracks has been raised from this cause.

Although *Micrococcus melitensis* can survive in dried dust for about a month, and it has been found possible to infect goats by feeding them on such infected dust, experimental infection by inhalation has not been fully demonstrated in regard to monkeys. In Part I of these Reports (pp. 46, 72) Horrocks detailed two experiments which indicated that "*Micrococcus melitensis*, when present in dry dust, is capable of being absorbed by monkeys"; but in the account of further experiments in Part IV (pp. 29, 31) the same observer stated that it had "not been found possible to infect monkeys with dust polluted with urine from Mediterranean Fever patients and then thoroughly dried. Goats, however, can be infected in this manner." As the enormous doses of strongly infected dust employed in these experiments are only occasionally capable of transmitting the disease, the probability of there being any habitual pathogenic property in the dust of urinals or rooms contaminated in the manner just mentioned, appears to be very remote. The possibility, however, cannot be disregarded; and although the *quantity* of the contagium may have been minute at any one point of time, it is likely that it has been constantly present in such places as Lower St. Elmo.

(d) The habits of the bulk of the Maltese population, as in Southern Europe generally, bring about a fouling of the ground with faecal and urinary excreta. The offices of nature are performed not only in private, but in public, places, advantage being taken of every nook and corner that offers. Around the barracks that are situated in the old fortifications there are so many ditches and secluded spots that the

* It is probable that the same thing happens, though it is not necessary, on the lower floor, in order to save trouble.

whole neighbourhood is sometimes a latrine; even within barrack limits it is often impossible to prevent this fouling of the ground by the native population. Floriana Barracks (including Notre Dame Ravelin and the intermediate ground) and Verdala, also all the Cottonera Lines, are instances in point. Outside Lower St. Elmo on the shore of the harbour, and in the Jews' Sallyport, the condition of the ground is particularly filthy. Wherever building operations are being carried on, as has been the case between the Porta Reale and Floriana Barracks during the past year, the fouling of the ground is also extreme. On one morning I counted 13 separate *dejecta* immediately outside the northern end of Floriana Barracks. It may be said that wherever troops are quartered in or near native towns or villages this fouling occurs in the immediate vicinity of their dwellings. Where they are removed from this undesirable propinquity it does not exist; as, for instance, at Manoel, Tigne, Ricasoli, Imtarfa, Gozo. The civil authorities seem powerless to put a stop to this nuisance; and of course the military have no control over ground outside barracks.* However, insanitary and disgusting as this condition is, it is not easy to prove any bad effects resulting from it in regard to Malta Fever, in view of the strong disinfectant action of sunlight that has been already mentioned. Only in such places (like the Jews' Sallyport) that are covered in, would the specific micro-organism retain its vitality for any considerable length of time. So also in the streets generally, though many corners are fouled, it may be assumed that the virulence of *Micrococcus melitensis* is soon destroyed by exposure to the sun. In the fields human excrement is frequently used as manure. Horrocks has found that *Micrococcus melitensis* may survive for 20 days in manured garden soil in the laboratory; but in the open fields, fully exposed to the sun, this would only be possible at some distance below the surface, from which situation it would not be likely to be dispersed about in the air, and inhaled or swallowed by any passer by.

The air of the streets of Valletta and other Maltese towns is, however, polluted from another source, viz., from the underground cellars, or basements, often used as dwellings, and in which there are often water-closets of the most defective kind. These closets are very scantily flushed with water, which has to be fetched by hand for the purpose, and, supposing any inmate of the basement dwelling is suffering from Mediterranean Fever, must undoubtedly be a source of danger to the other occupants; and not only to the occupants, but to the passers-by in the streets above. The effluvia that rise from these basements are often very offensive, and obviously excrementitious: as

* There is, I believe, one exception to this. I was informed that the troops occupying Lower St. Elmo are charged with the duty of keeping clean the Jews' Sallyport, which is used as a latrine by the natives of the neighbourhood.

these places are dark, and never penetrated by the sun's rays, there is no reason to suppose that *Micrococcus melitensis* would lose its virulence in a hurry in such a situation. Alternations of temperature cause currents, upwards and downwards, from these basements; and it is within everyone's experience that the current upwards is sometimes (like the effluvium) of considerable strength, and quite able to carry up micro-organisms from the closet below to the street above. In this way the men occupying barracks such as Upper and Lower St. Elmo, which cannot be approached except by passing along streets having basement dwellings of this kind, are more liable to aerial infection than the occupants of barracks such as those at Pembroke and Imtarfa, situated away from such streets and dwellings.

(e) In the late Captain Hughes's treatise on Malta Fever there is a strong body of evidence in regard to the association of fever outbreaks with "insanitation"; implying by this contamination of the air of the barrack or dwelling with emanations from drains, cesspits, etc., or putrefying organic matter, or polluted soil. Fifteen separate outbreaks of greater or less extent are carefully described in which the connexion certainly appears to be one of cause and effect. I made particular enquiry of 187 patients suffering from the disease, as to whether they had been conscious of any insanitary condition, or "bad smell," in or near their quarters, which might seem to be connected with their illness. The information gained was disappointingly meagre. In only six cases was there any idea, from the patients' side, of any connexion between "bad smells" and their illness. In one case a w.c. in the officers' mess, where the man was employed, had been frequently stopped up and offensive. In two cases the regimental latrines sometimes became choked, and the men had to clear them, which was a disagreeable job. A man employed at the officers' mess in the Inquisitor's Palace, slept on the ground floor, where there were often bad drain smells.* One man (and one only) complained of the bad state of the latrines in Cottonera Lines. One serjeant complained of a bad smell in his "bunk," which was very imperfectly ventilated (Lower St. Elmo). This testimony is of very slight importance one way or the other; all one can say is that there does not appear to have been any notable or widespread offensiveness in any of the barracks sufficient to excite attention. This is, after all, what one would expect in barracks, where the dwelling rooms are quite disconnected from the latrines and drainage. Only in the old fortress barracks, and in hospitals, are these conditions reversed. But during the last 10 years very great improvements have been made in the condition of these old barracks, and the insanitary conditions detailed by Hughes are not, to the best of my belief, now existing in any quarters occupied by troops

* This quarter (and the mess building) was evacuated shortly afterwards. When I examined it, I could find no defect in the drainage arrangements.

in Malta. The evidence collected by him is, in my opinion, strongly in favour of a causal connexion between Mediterranean Fever outbreaks, and the laying on of excrementally polluted air to dwelling rooms; but I have not been able to gather any similar evidence that would in any way explain the incidence of the disease during the past year amongst the troops.

§ 6.

Having reviewed the influence of water, food and air as channels of infection in Mediterranean Fever, with on the whole a negative result, that is, without having succeeded in tracing any definite relationship between its mode of prevalence amongst the troops and the existence of conditions pointing to probable infectivity of these media, we are now led to the consideration of what appear to be the only other alternatives, viz., direct or semi-direct contagion, and the agency of some biting insect.

As to direct contagion, Hughes, writing in 1897, dismisses the question very shortly. "Patients suffering from other diseases, occupying beds next to cases of undulant fever, do not develop this fever, nor do the military sick attendants in fever wards suffer more from this fever than those working in other wards, or so much as soldiers in many of the barracks in Malta who have not entered the hospital previous to the onset of their attacks."

The following table is extracted from a paper by Capt. J. C. Kennedy,* and shows the prevalence of Malta Fever amongst patients and orderlies at Valletta Hospital, as compared with the garrison in Valletta, for the years 1897—1904. The figures are ratios per 1000.

	Valletta Garrison.	Valletta Hospital patients.†	Valletta Hospital orderlies.
1897	42·11	11·05	80·00
1898	22·78	29·99	168·63
1899	22·54	32·00	34·48
1900	26·08	6·44	54·05
1901	43·11	45·75	121·21
1902	15·90	34·18	48·78
1903	67·31	24·53	50·00
1904	45·42	14·43	169·23
Average ...	36·23	24·79	92·4

* 'Journal R.A.M.C.,' May, 1905.

† Cases that have been diagnosed as Malta Fever within 20 days after admission, and cases that have been changed from S. C. Fever to Malta Fever after admission, have been excluded. Also cases that have been admitted from outside, but which may have contracted the disease inside, hospital are not included.

Captain Kennedy points out that venereal patients, and patients suffering from injuries, were much more liable to contract the disease than others, the ratio being 3·31 per 1000 venereal admissions, 2·42 per 1000 admissions for injury, and only 0·76 per 1000 admissions for all other diseases. He explains this by the facts that these patients spend a longer time in hospital, on the average, than any others (except Malta Fever); and that they are all treated in one ward, 20B, which is in communication with, indeed is part of the same room as, other wards containing Malta Fever patients. In 1905, 11 cases have apparently been contracted in Valletta Hospital, of which 8 were staying in 20B Ward and 2 in 20A Ward; the ward in which the remaining case stayed is doubtful. At Cottonera 10 cases apparently contracted the infection, of which 3 were inmates of wards in which the fever cases were treated. As regards orderlies, Kennedy states that of the 11 who contracted the disease at Valletta in 1904, 8 were doing duty in 20A, 20C, and 37 wards containing Malta Fever patients. In 1905, of the 19 at Valletta who were attacked, 11 were employed in the fever wards. At Cottonera 5 out of 7 cases amongst orderlies were similarly employed; as were the two cases of R.A.M.C. at Citta Vecchia.

Now, leaving on one side for a moment the case of the orderlies, who are exposed to various possible sources of infection, what is the most probable explanation of the occurrence of these cases of infection in patients who are confined to the hospital precincts, and in some instances to their beds? At both Valletta and Cottonera Hospitals the drinking water is above suspicion, the milk has been "pasteurised" since the middle of 1904, and the wards are absolutely free from any kind of contamination from sewer air, or latrine air, or urinal air. Whatever the sanitary shortcomings of the "Long Ward" in Valletta Hospital may be, it is certainly not exposed to any danger of this kind; neither are the other wards in this hospital, nor any of those at Cottonera. Of course, patients who are able to get up make use of the latrines and urinals of the hospital; but in neither of these hospitals has there been any failure in the water supply to latrines, leading to insufficient flushing, nor has there been any reason, even the slightest, to suspect that drain effluvia gain access to the latrine or closet chamber. The latrine for 20B Ward is certainly old and defective, and a considerable waste of water results on account of the defective fittings; also the latrine and urinal for No. 37 has a rough floor, which requires concreting. But though these conditions are insanitary and undesirable, they cannot be reasonably held to be causative of Mediterranean Fever.

The condition that appears to be the most probably effective in the causation of these hospital cases is the presence in the wards of a large quantity of disease-producing material in the bodies of the patients

themselves. It is known that the specific organism is present in the blood, and is excreted in the urine; it is possibly excreted in faeces, but up to the present has not been demonstrated in the breath, saliva, or perspiration. Transmission by direct contagion is therefore not theoretically probable; by indirect or semi-direct contagion through clothing soiled with excretal discharges it is not improbable in the nature of the case, although hitherto there has been no proof of this mode of spread. The position, however, is not unlike that of enteric fever, which is now considered (in fact, may be said to have been proved) to be spread by means of "contact," *i.e.*, close association. Presumably this happens by infective urine or faecal matter fouling the skin or clothing of the patient, and then becoming disseminated through the air, and inhaled; or finding its way into articles of food or drink, and being swallowed. We have the authority of Koch for the opinion that transmission of enteric in this way is its most important mode of propagation. A few years ago this would have been considered most unlikely, but proofs have been accumulating. I do not see that there is any essential difference between the position as regards enteric fever transmission and Mediterranean Fever transmission. Where there is a large quantity of the infective material accumulated in one place, *i.e.*, in a hospital, there the likelihood of its spread is the greater. That this spread occurs but very seldom is because the obvious precautions usually taken are sufficient; but when the number of cases (*i.e.*, quantity of specific poison present) is largely increased, it may probably happen that the precautions are not increased *pari passu*, because the labour involved increases out of all proportion to the working power present. A patient severely ill may pass involuntary evacuations twice or three times in the night. There may be (and have been) two or more such cases in the same ward; obviously the risk of dissemination of infective particles becomes much increased when this occurs. Even with the best methods of disinfection in every detail, of the person, of the clothes, of the evacuations, there must be a chance under such conditions of infective material being spread about. This seems to be a mode of propagation that cannot be excluded; it is applicable to the other occupants of the wards, and especially applicable to the actual attendants on the fever cases.

With regard to the behaviour of the epidemic among the troops in barracks, from the preceding part of this section it appears that neither water, nor food, nor air contaminated with drain emanations will explain the incidence of the disease; the one fact that stands out most clearly is that the fever has occurred in a number of small outbreaks, almost strictly localised in some place, or limited to some small body of men. Examples of this have been instanced in the case of G and H Companies, Essex Regiment, at Lower St. Elmo; A Company, Royal Dublin Fusiliers, at St. George's; the men of the Royal West Kent

Regiment that occupied the Old Barracks, Floriana. In each of these instances, where several cases of fever occurred in the same room, or same set of rooms, there was an appreciably larger quantity of infective material in those rooms, than in the barracks generally; the more there was of it present, the more likelihood would there be of the infection spreading.

There is one condition, common to Lower St. Elmo and Floriana Old Barracks, that would presumably be of importance in aiding this spread of infection. The rooms are casemates, most inadequately ventilated. If it be granted that the infective material is disengaged from the bodies of persons suffering from the disease, no better place could be found for its accumulation from day to day and night after night than a casemate such as those in question. It is extremely improbable that a thorough change of air ever takes place in these cavernous chambers. It is quite impossible that any thorough change should be effected frequently. The construction of the rooms and their size prevent it. I do not think it too much to say that the Long Ward in Valletta Hospital is in similar case as regards change of air. Though very large and lofty, the thorough change of the contained air is very difficult to effect: and as the upper windows have not (to the best of my belief) been fully utilised as outlets, I consider that there has been an accumulation of infective material in the air of this ward from day to day and night after night.

In regard to the barrack-rooms at St. George's that were so much affected (A Company, Royal Dublin Fusiliers), nothing can be said against their ventilation. But the bedcots are crowded together, so that only about 12 inches separate each pair of beds, and there has therefore been concentration of the persons, and consequently of the infective material. It may be asked in this, as in the other cases, where many barrack rooms are similarly circumstanced, why some should be affected and not others. The reply would be that it is necessary that the poison should be introduced, and probably introduced in some notable quantity; having once been introduced, the conditions mentioned would naturally favour its spread.

There are two main difficulties to be met in adopting this theory, or explanation, of the prevalence. One arises from the fact that *Micrococcus melitensis*, though often sought for, has not been found either in the air of the Valletta Ward or in the dust collected from it, and from the Cottonera Wards. The other is that it has not been found possible, so far, to infect monkeys with urine-infected dust. It must be admitted that these are substantial difficulties in the way of this explanation.

§ 7.

In regard to the question of transmission by fomites, the experiments of Horrocks, who found that *Micrococcus melitensis* could be

recovered from khaki cotton, khaki serge, and blankets up to the 80th day; and of Shaw, who recovered it from blue serge up to the 78th day, show that this form of dissemination has practical importance. The necessity for disinfection of clothing, etc., is fairly obvious. The procedure that has been carried out has varied in the different corps stationed in Malta, as appears from the following statements obtained from the regimental authorities:—

Royal Garrison Artillery (Upper St. Elmo), 65th Company.—In the earlier part of the year the kit and bedding of men admitted to hospital were placed in the company store until instructions were received from the medical authorities that they should be sent to hospital for disinfection. Since the middle of August, in the case of all men admitted with "fever," the kit and bedding have been put on one side in the "Old Magazine," awaiting instructions as to their disposal.

96th Company. It has always been the custom to put on one side the kit and bedding of all men admitted to hospital. When the case was declared to be "fever," the whole kit and bedding has been sent to Cottonera Hospital for disinfection.

Tigne, 99th Company. The kit and bedding of all men admitted to hospital have been placed in company store; on receipt of instructions from the medical officer in charge of the district, either "kit," or "kit and bedding," have been sent to the lazaretto for disinfection.

1st Company. Same as in 99th Company. In about half the cases, "bedding" only has been specified, and the "kit" has not been disinfected.

102nd Company. The kit and bedding of all men admitted to hospital are placed in company store. In infectious cases a paper of questions is sent by the medical officer to the commanding officer; one of these has reference to the kit and bedding; if they have not been disinfected they are to be sent to the lazaretto for disinfection. It may be 10 days after a man has been admitted to hospital that instructions arrive as to disposal of kit.

Ricasoli, 5th Company. When a man goes to hospital, his kit and bedding are taken into the company store; if instructions come from the hospital authorities his "kit" is sent to Cottonera for disinfection, but *not* the "bedding."

63rd Company. Same as in 5th Company, except that the "bedding" is sent to be disinfected but *not* the "kit."

100th Company. Same as in 5th Company: the bedding is *not* disinfected.

Hampshire Regiment (Verdala).—When a man reports sick, his kit and bedding are brought out of the barrack room and placed in the company store. If he is not admitted to hospital, he takes his kit and bedding back to the barrack room. If he is admitted, his kit and bedding are stored in the company store, the blankets being all stacked together in order, the sheets all together, and the mattresses all together. There is no certainty that a man receives the same blanket on discharge from hospital as he handed in when admitted. Sheets and pillow slips are washed. No difference is made between "fever" cases and others. Any dirty clothing in the kit bag remains *in situ*. In infectious cases, instructions come from the hospital authorities to the commanding officer that "kit and bedding" are to be sent to hospital for disinfection on some named date. Some days, a week or more, may elapse (after the man's admission) before these instructions are received.

Lancashire Fusiliers (Lower St. Elmo).—The kit and bedding of men admitted to hospital are stored in the company stores (the prison cells being used for this purpose). The hospital authorities notify (after an interval of some days) when the kit and bedding are to be sent for disinfection. There are no means of keeping

separate the kit and bedding of "suspected," i.e., fever cases: but if any man is admitted with "fever" his kit and bedding are sent for disinfection on the first Tuesday or Friday that follows.

Essex Regiment (Imtarfa).—The bedding and blankets of men admitted to hospital are sent for disinfection when so ordered by the hospital authorities, but the kit remains in the man's kit bag, unless obviously dirty, in which case it is sent to the wash. Kits and bedding are stored in parts of barrack rooms appropriated for the purpose, there being no space for their disposal in the rooms labelled "company store," which are little better than cupboards.

Royal West Kent Regiment (Floriania).—Formerly the bedding of men admitted to hospital used to be left in the barrack room. Early in the summer of 1905 the practice commenced of sending the bedding of *all* cases admitted to hospital, to be disinfected, so as to be on the safe side. The kit has been kept in company store in the two kit bags, and has not been sent for disinfection; nor have the dirty articles of clothing been washed, until the man's discharge from hospital.

Royal Dublin Fusiliers (St. George's).—Until the latter part of August only the bedding of cases of Mediterranean Fever was sent to hospital for disinfection; cloth articles of clothing were exposed to the sun and brushed; khaki, under-clothing, etc., was left in the kit bag *in situ* in company store. Since the beginning of September everything has been sent to be disinfected.

Rifle Brigade (St. Andrew's).—Same as Dublin Fusiliers.

From the above account it is obvious that the disinfection of the clothing and bedding of Mediterranean Fever patients has been, during the greater part of 1905, far from complete. The want of uniformity in procedure is remarkable. Assuming that infective material may be present in soiled sheets, blankets, shirts, trousers, etc., there must have been opportunity for dissemination amongst the men of the same company, or unit, in many cases. In those instances where bedding (including blankets) has not been disinfected, it has been possible for the blankets or other articles, that have been given into store by one man, to have been taken into use by another man, as it is not the general practice to label the blankets, etc., individually; the company storeman would return to a man on discharge from hospital the same blankets that he had deposited in the store on admission, if he knew which they were; but this would not always be the case. In the instances when bedding has been sent to be disinfected on instructions being issued to this effect, there was generally an interval of a week or 10 days before the instructions arrived; and during this time infection might be transferred to other blankets or bedding in contact with the infected articles. In those cases where the kit was not sent for disinfection, when the kit-bag was subsequently opened out, and any dirty shirts, etc., sent to the wash, there would be a chance of disseminating infective material. It is to be noted that, when a man goes to hospital, he generally puts on a clean shirt, etc., the dirty shirt, etc., going into his kit-bag. There is therefore some presumption that infective material might be present. When the washing day came round, sheets and pillowslips would be sent to the

wash ; the dirty shirt, etc., might be sent to the wash, but it would more likely remain in the kit-bag until the owner came out of hospital.

As there were 487 cases of Mediterranean Fever during the period under review, and therefore 487 bundles of bedding and kit to be handled, one would expect that if these articles were infective, the "company store men" who handle them would show some increased liability to contract the disease. But in only one case could I ascertain that a storeman had been attacked. This was Private Burch, Essex Regiment, who was admitted to hospital on June 3, 1905. He stated that it was his duty to handle the clothing and bedding of Mediterranean Fever patients, and that sometimes this had been offensive, especially after having been fastened up in a bundle for some time.

It would seem to be probable that infection might be conveyed through infective fomites ; and if this be the case, the measures of disinfection that were taken—up to September—could not be supposed to prevent this dissemination, looking at the whole question broadly. If a comparison however be made between the severity of incidence in the different corps and the method of treatment of presumably infected kit and bedding, it is seen that there is no general relation between the completeness of the disinfection and the severity of the attack ratio.

	Attack ratio per 1000.
Kit and bedding disinfected—	
R.G.A., 65th Company, Upper St. Elmo	83
" 96th " " 	69
" 102nd " Tigne	32
Lancashire Fusiliers, Lower St. Elmo	63
Hampshires, Verdala.....	27
Kit disinfected, not bedding—	
R.G.A., 5th Company, Ricasoli	34
Bedding disinfected, not kit—	
R.G.A., 63rd Company, Ricasoli	18
" 100th " " 	42
Essex, Imtarfa	88
R. W. Kent, Floriana.....	45
Bedding disinfected, kit sunned and brushed—	
Dublin Fusiliers, St. George's	46
Rifle Brigade, St. Andrew's	54
Sometimes kit, sometimes bedding, sometimes both, disinfected—	
R.G.A., 1st Company, Tigne	68
" 99th " " 	54

Systematic and complete disinfection has been carried out in all cases, I believe, since the middle of September, 1905.

§ 8.

The discovery by Horrocks and Kennedy of *Micrococcus melitensis* in considerable numbers in the stomach contents of two species of mosquito (*Culex pipiens* and *Stegomyia fasciata*) indicates that transmission through the medium of biting flies is a possible mode of propagation. The arguments in favour of direct contagion or aerial transmission would apparently hold good equally in regard to mosquitoes, as carriers of infection, in places such as Malta, where they abound. Granted the presence of infective material in a ward or barrack room, in the shape of hospital patients or ambulatory cases of the disease, transference to the healthy in this way becomes easily intelligible; the numerous localised outbreaks are explicable on this hypothesis, as reasonably as by direct or semi-direct contagion. The only contribution that I am able to offer to this part of the subject is to mention that, of 97 patients from whom a *definite* statement was obtainable as to their experience of mosquitoes, 31 asserted positively that they have never, or practically never, been bitten at all; 18 stated that they had been bitten very slightly; while 48 admitted that they had been bitten a good deal. Without attaching much value to these statements (which, however, I believe to be accurate as far as they go), bearing in mind the rarity with which *Micrococcus melitensis* has been found to be present in the mosquito (four times in 896 individual mosquitoes), the chances seem to be very much against the entrance of the germ into the body having taken place in this way in the case of the 49 men who were either bitten but very slightly or not at all. But the number of men dealt with is insignificant.

SECTION IV.—CONCLUSION AND RECOMMENDATION.

The chief facts ascertained in this enquiry into the prevalence of Mediterranean Fever amongst the troops in Malta have been summarised in Section III, §1; the various modes of propagation of the disease that have been suggested by different observers have been considered in order, and, on the evidence of the facts ascertained, a negative conclusion as to their ability to explain the behaviour of this epidemic has been arrived at in regard to transmission (1) by water, (2) by milk or other articles of food, (3) by air contaminated with excremental (fæcal or urinary) effluvia; transmission (4) by direct or semi-direct contagion, or (5) through the agency of mosquitoes appears from the evidence to be more probable than in any other way; it is difficult to separate these two modes of dissemination, the one from the other, under the circumstances existing in Malta, and provisionally I think they may be considered together.

Fully admitting that no proof has been afforded in support of this opinion, I still consider that there is a high degree of probability attaching to it, and one quite sufficient to warrant the adoption of certain measures of prevention or precaution.

Whatever view be taken of the mode of propagation, the fact is undoubted that certain barracks have suffered much more than others; among these are Lower St. Elmo, Upper St. Elmo, and the old barracks at Floriana.

If considerations of economy and the maintenance of the health of the troops were the only things to be considered, probably the cheapest and most healthful course to pursue would be to evacuate these barracks altogether. If military considerations render this impracticable, I consider that an efficient alternative would be afforded if the following procedure were carried out:—

(1) Let it be recognised that these casemate barracks are entirely exceptional in their construction and need to be specially dealt with; the occupancy should be reduced from the present numbers (calculated on a cubic space of 600 cubic feet or less per head) to one which would allow 750 cubic feet *at the very least* per head, as is now admitted to be necessary in the case of all new barracks in the command. No height above 12 feet should be reckoned as available for ventilation in the calculation of this space.

(2) During the summer months tentage for 25 per cent. of the occupants of Lower St. Elmo should be drawn (as is the case in all the other barracks in Malta, except Imtarfa and Pembroke), so that the condition of the barrack rooms at night may be alleviated as much as possible in regard to heat, stuffiness and organic contamination of the air.

(3) As even under the best possible conditions, change of the air in these casemate barracks is very difficult, and accumulation of impurities on the walls and ceilings therefore much greater than in barracks of ordinary construction, all walls and ceilings of rooms and passages should be limewashed at frequent intervals, say, once a month; this would ensure the removal of dirt, the extermination (for the time) of mosquitoes, and, for practical purposes, would be a disinfectant measure. As the work could be done by the troops, the expense would be insignificant.

(4) There is sufficient evidence to warrant a presumption, at any rate, of localised infection, or semi-direct contagion: in the event of two cases of Mediterranean Fever occurring in the same room within a fortnight, the barrack room should be evacuated and limewashed, the men being accommodated in tents for the time; after this has been done, the room might be re-occupied; but if another case occurs within a fortnight of re-occupation, it should be again evacuated, and the body of men isolated as far as possible.

(5) If in any company, or small detachment, several cases occur in quick succession (*e.g.*, two in one week, or three in a fortnight) this body of men should be regarded as infected. They should be placed under canvas, or transferred elsewhere; the measures suitable for each individual outbreak of this kind can be decided on according to the special circumstances of each case. The remarkable freedom from fever experienced by the Gozo detachments of the Essex Regiment (which regiment suffered so severely at Lower St. Elmo) indicates that, if any small infected body of troops were removed from their surroundings to a detached spot (*e.g.*, Gozo, or Mellicha, etc., etc.), and scrupulous attention paid to their sanitation in every detail, the infection may be expected to die out. Once it is evident that a body of men is infected, the sooner the move is made the better; probably a very short distance would suffice; but there must be no overcrowding, and every detail of sanitation must be carefully attended to. The rooms evacuated should be fully disinfected with formalin or other disinfectant.

(6) The above recommendations refer especially to the three old barracks that have suffered severely from Mediterranean Fever; there are other old barracks of similar defective construction to which the same recommendations are applicable, though Mediterranean Fever has not been especially prevalent in them during the past year. Such are St. James Cavalier, Salvatore Counter Guard, St. Francis Barracks, Marsamuscetto, Old Laboratory, all the barracks in the Cottonera Lines, and Fort Chambray, Gozo. In all of these the recommendations as to 750 cubic feet of space per head, 12 feet of height only being reckoned for the calculation of space; frequent limewashing, and evacuation and disinfection on the occurrence of Mediterranean Fever, equally apply. Although they have not suffered in 1905, their defects are such that a prevalence of the disease is to be feared, if the infection be introduced in sufficient amount. Tentage is already authorised to be drawn for these barracks in the hot weather.

(7) In the modern barracks in Malta, which are so satisfactory in their construction and general sanitary conditions, the above recommendations do not appear to be necessary; but the principle of a stitch in time equally holds good; any succession of cases in a barrack room would indicate the advisableness of evacuation, disinfection, and isolation.

(8) The defects in the supply of water for sanitary purposes throughout the Cottonera district (including Verdala), at St. James Cavalier and at St. George's require immediate attention. The provision of an ample supply of sea water, or No. 2 water, in order to flush the latrines and drains properly is such an obvious necessity, that it is strange that a recommendation to this effect should require to be made. Increased pumping power appears to be what is wanted;

but this is a matter for the Royal Engineer department to determine. The necessity is urgent. Latrines should be flushed at least three times a day, and four times a day if water is available.

(9) The management of urinals has not been properly carried out: systematic application of "heavy oil," or some efficient substitute (such as colza oil and tar, as used at Imtarfa); the omission of water flushing for the stalls (which, strangely enough, has very generally been used along with the oil treatment); but the careful washing of the floors of urinals, and flushing of the urinal drains with water; these are the measures indicated.

(10) Where the dry-earth system of excreta removal is still in use, a removal of three times, instead of once, in the 24 hours is recommended: this will necessitate the provision of suitable receptacles for the pail contents. The present system is too barbarous and offensive to be tolerated. There is no reason why what is strictly forbidden in India should be universally permitted in Malta: I refer to the retention in the latrines of pails, full of filth, for the greater part of the 24 hours. The nearest approach possible to the *immediate* removal system (according to the Indian fashion) should be made. It is to be hoped that the dry-earth system will, before long, be completely abolished in Malta, except for temporary camps.

(11) If the above recommendations (8) to (10) are carried out, it is to be expected that the contamination of the air of barracks generally by excremental emanations, also the risks due to disease conveyance by flies, will cease; until this is the case no barrack can be considered to be in a good sanitary condition, whether in reference to Mediterranean Fever or any other infectious disease. There is one special preventive measure directed against Mediterranean Fever infection that has been carried out during the past year, viz., the boiling or "pasteurising" of milk for the troops. This requires to be continued in the strictest possible manner. As regards the married families, for whom this is more important than for the troops, it might be feasible to provide Aymard sterilisers to treat the milk supply of the large married quarters centrally, and therefore more effectually. If this is impracticable, special instruction and warning should be continually given, not only to new arrivals, but to all the married people, as to the necessity for sterilising goats' milk, or else substituting condensed milk for it.

(12) As success in dealing with this disease, as in other infectious fevers, will probably largely depend on stopping the beginnings of an outbreak, i.e., carrying out the principle of *Obsta principis*; perhaps the most important thing of all is to find out as early as possible when and where anything like an epidemic prevalence is commencing. The existing arrangements for arriving at a diagnosis are satisfactory; if, however, this could be expedited, it would be very desirable that it

should be done. But what is required, in my opinion, is some method of tracking out the cases as soon as they occur; not waiting for a final diagnosis, which cannot be arrived at for perhaps 10 to 14 days, but, examining the patient, his surroundings, habits, movements, etc., etc., whenever there is even a *probability* of the case being one of Mediterranean Fever. Information can be easily obtained at the time, which afterwards can only be got at with difficulty and labour, or not at all. The existing establishment of medical officers is not, in my opinion, adequate for this work, they being all fully employed, as it is, especially during the summer months, when the sickness is greatest. I consider, and recommend, that two extra medical officers be employed, to give their whole time to this epidemiological investigation work, over and above the work that has been hitherto, and is now being done in the laboratories and in the various hospitals. The tracking out of the early cases by a skilled observer ought to lead to important results, in the way of ascertaining modes of infection, and consequently the carrying out of effectual measures of prevention. I do not think that one medical officer would be sufficient, the ground to cover is too extensive, and the investigations must be undertaken without delay in each case; in the summer months at least two officers would be busily occupied every day. They should be employed for this purpose only.

(13) Immediate and effectual disinfection of clothing and bedding of men admitted to hospital with Mediterranean Fever, and of patients in hospital suffering from this disease, should be carried out as a matter of course. To be effectual the disinfection must be complete, not as was formerly the case.

(14) Isolation of Mediterranean Fever patients when in hospital is indicated. The difficulty is to carry it out at Valletta Hospital.

(15) The management of the Long Ward at Valletta Hospital, so as to ensure proper change of air, is a very difficult problem to solve. I am convinced that much more ventilation, a much more frequent and thorough change of air than has hitherto been the case, is required. More advantage might be taken than was the case last summer, of the existing windows in the upper storey. A new hospital is urgently required, as has been insisted on for about 30 years past.

XIV.—FURTHER MOSQUITO EXPERIMENTS.

(Continued up to the end of January, 1906.)

By Captain J. CRAWFORD KENNEDY, R.A.M.C.

(Received February 5, 1906.)

From the middle of October, 1905, until the number of mosquitoes obtainable was too small to be of experimental use, the following experiments were carried out:—

Experiment A.—Mosquitoes were collected from the mosquito nets of Malta Fever patients and the wards of Citta Vecchia Hospital; they were all without exception *Culex pipiens*, and had fed well during the previous night on the patients; they were placed in a cage (marked "A") over water and then given the opportunity of feeding each night on a monkey. A supply of mosquitoes was received daily, and from October 13 to November 12 between 600 and 700 were collected. The monkeys bitten were Nos. 93, 22, and 33.

Monkey No. 93.—This animal had been used by Major Horrocks during the summer in order to try to infect it by means of dust collected from various parts of the island and blown down its nostrils. The last time this experiment was performed was early in July, so that there was a clear interval of at least $2\frac{1}{2}$ months during which there was not a trace of a reaction to the *Micrococcus melitensis* in its blood.

The mosquitoes in Cage A were placed on this monkey nightly (with a few exceptions) from October 16 to October 31, in all 11 times; and I consider that about 500 mosquitoes had a chance of biting during that time. The animal's blood was tested for reaction to *Micrococcus melitensis* on October 16, 22, and 29, and November 5, but with no result. On November 12 there was a faint reaction in a 1/10 dilution. On November 19 a faint reaction was also perceptible in 1/10 in half an hour, and this became quite distinct in one hour. On November 25 this faint reaction had disappeared; nor was any reaction obtained on the following dates: December 2, 10, and 17. On December 31 a faint reaction appeared again. On January 7, 1906, there was a more or less complete reaction in 1/20 in half an hour. On January 10 and 12, the same; but on standing for $1\frac{1}{2}$ hours the reaction was complete in 1/80 and incomplete in 1/100. On January 21 the reaction had not increased and, fearing that it might again disappear, I chloroformed the animal on January 24 and made a careful *post-mortem* examination.

The glands in the femoral and the axillary regions were enlarged and more numerous than usual. The spleen and other internal organs were normal.

Cultures on plates were made from the following organs :—

Spleen—entirely cut up and impressed on five plates.

Liver—two plates.

Kidney—two plates.

Glands, mesenteric—four plates.

„ femoral—five plates.

„ axillary—four plates.

Micrococcus melitensis was recovered from all the plates made from the axillary and the femoral glands. All the other organs were sterile. The microbe was present only in small quantity ; the axillary contained more than the femoral glands.

Monkey No. 93 was, therefore, infected with *Micrococcus melitensis*. The fact that the microbe was localised so completely in the glands and had never reached the internal organs, probably explains the unsatisfactory nature of the agglutinative reaction.

The observation of the presence of a faint reaction 12 days after the last exposure to infection is important, (1) as proving the presence of the microbe in the body at that date, and (2) as showing that it had been there long enough to cause the formation of agglutinins ; otherwise the long incubation period before a complete agglutination was obtained might cast some doubt on the experiment.

The animal never had any rise of temperature.

There was no chance of the monkey becoming infected in any other way than by the bites of the mosquitoes. The conditions under which it lived were as follows :—

For the whole summer it lived in one box shut off from any possibility of contamination from infected animals by waterproof partitions. It was situated at the end of a row, so that there was no animal on its left side. The box on its right was always occupied by an uninfected animal. No animal contracted the infection accidentally during the year. On December 14 it was removed along with three other uninfected monkeys (mosquito experiments) to a mosquito-proof room in the Lazaretto which had previously been carefully disinfected and whitewashed. These three other monkeys show no sign of infection.

Monkey No. 22 was only bitten three times and was never infected. It died from tuberculosis.

Monkey No. 33 arrived in October from Bombay, and was kept under observation till November 2. On that date and regularly till December 11 the mosquitoes in Cage A were fed on it. The cage was put on 27 times altogether, and I estimate that about 100 mosquitoes had a chance of biting.

The monkey has up to date never showed signs of reacting to *Micrococcus melitensis*.

Experiment B.—Mosquitoes caught promiscuously and presumably

uninfected were placed in Cage B, with water in which *Micrococcus melitensis* was held in suspension. They were fed regularly on Monkey No. 36 between November 11 and December 11.

Monkey No. 36 has never up to date showed signs of reacting to *Micrococcus melitensis*.

Experiment C.—Freshly-hatched *Culex pipiens* were collected in Cage C, and kept alive by feeding them alternately on an infected and an uninfected monkey.

Between November 3 and December 11 they were fed on Monkey No. 101 twice, on Monkey No. 27 14 times (both these monkeys were infected and contained *Micrococcus melitensis* in their blood), and on Monkey No. 14 (uninfected) 12 times.

Monkey No. 14 has up to date never shown signs of reacting to *Micrococcus melitensis*.

SUMMARY.

I. Monkey No. 93 became infected with *Micrococcus melitensis* as the result of bites from mosquitoes (*Culex pipiens*) which had fed on patients suffering from Malta Fever.

II. An attempt to infect a monkey by means of bites from artificially infected mosquitoes failed.

III. Attempts to convey infection from an infected to an uninfected monkey by means of freshly-hatched mosquitoes (*Culex pipiens*) were unsuccessful.

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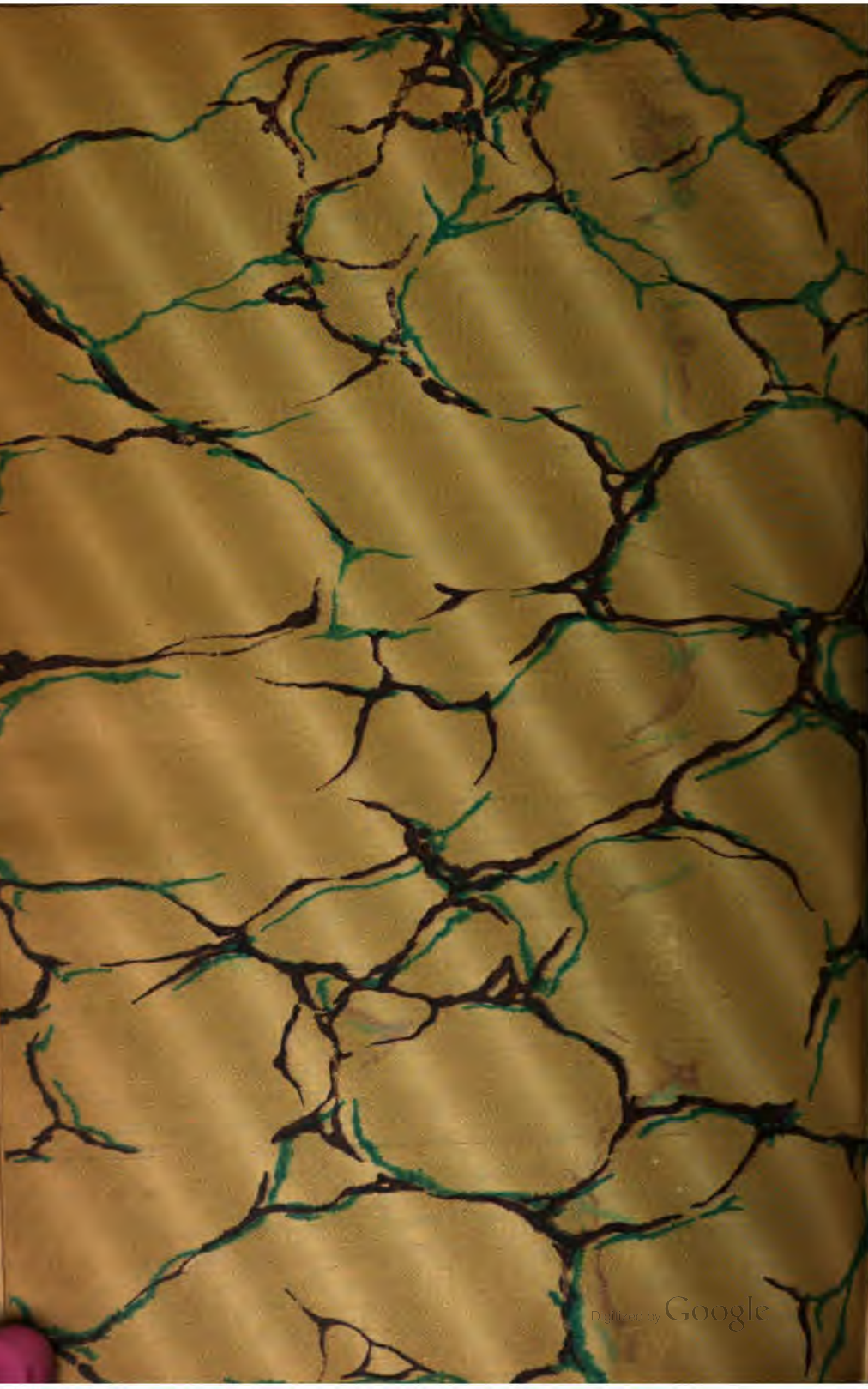
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